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THE
DEVELOPMENT OF PHRYGANIDS,

WITH A

PRELIMINARY NOTE ON THE DEVELOPMENT
OF BLATTA GERMANICA.

AN INAUGURAL DISSERTATION TO OBTAIN THE DEGREE OF DOCTOR
OF PHILOSOPHY, SUBMITTED TO THE UNIVERSITY OF LEIPZIG.

BY

WILLIAM PATTEN,
OF BOSTON.

Reprinted from the 'Quarterly Journal of Microscopical Science,' 1884.

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The Development of Phryganids, with a Preliminary Note on the Development of Blatta Germanica.

By

William Patten,
Of Boston, U.S.A.

With Plates XXXVIA, XXXVIB, and XXXVIC.

I. GENERAL REMARKS.

THE¹ eggs of this insect (*Neophalax concinnus*) were obtained in great quantities from the muddy bottom of a slow running stream in the neighbourhood of Boston, Massachusetts. About 150 eggs are embedded in a spherical, vesiculated, semi-transparent mass of gelatinous substance, which either lies freely on the top of the mud, or is more or less covered by it according to the length of time it has been deposited. Very rarely it is attached to some stick or stone.

The eggs are found in the greatest abundance in water four or five inches deep, and where there are a great many twigs lying on the bottom and projecting above the surface. These twigs would undoubtedly afford an opportunity for the females to crawl below the water and deposit their eggs in the manner described by McLachlan.² I³ have never seen them myself

¹ A part of the following investigations were carried on under the direction of Dr. E. L. Mark, at the Museum of Comparative Zoology at Harvard University, Cambridge, Mass., the remainder under the instruction of Professor R. Leuckart in the Zoological Laboratory at Leipzig.

² 'Monographic Revision and Synopsis of the Trichoptera of the European Fauna,' Robert McLachlan, 2 vol., 1874—80.

³ The following description applies to Phryganids unless otherwise stated.

ovipositing in this manner, although I have watched them a great many times as they flew over the surface of the water in the neighbourhood of the place where the eggs were found in the greatest abundance.

The eggs are probably dropped by the insect as it alights on any of the numerous twigs projecting above the surface, and since they readily sink there is no necessity of the females going beneath the water to oviposit, as is the case with those *Phryganidis* which fasten their eggs to the under side of various water plants.

Rathke, Zaddach, and McLachlan state that *Phryganids* lay their eggs, arranged in a single layer and enveloped in a transparent, gelatinous substance, on the under side of various water plants, such as *Hydrocharis morsus ranei*, *Potomagaton*, and *Nymphæ*. It will be observed that *Neophalax* deposits its eggs in an entirely different manner, and in localities where such water plants are not to be found.

As far as could be observed, the only substance of a vegetable nature available to the larvæ for food was the mass of decaying twigs covering the bottom, and which had fallen from the thickly overhanging trees. Unfortunately the stomachs of the larvæ were not examined, so I cannot say with certainty what the exact nature of the food is. It is very probable that the more developed larvæ feed principally upon dead vegetable matter, since I have found such twigs fairly bristling with the larvæ, and at the same time showing many traces of having been gnawed by some insect. The young larvæ, however, devour *Vorticellæ* and various other *Infusoria* with avidity.¹

The eggs have a strong odour of "musk," which is still perceptible after they had been hardened by heat and preserved in alcohol for several months, and even more so after they had been softened a half hour or more in water.

The seat of this odour is in the gelatinous envelope since it was not noticeable in the eggs, but, on the other hand, was very strong in the gelatine which had been separated from the eggs.

¹ It has long been known that *Phryganid* larvæ are in general carnivorous; *Neophalax* is thus an apparent exception to this general rule.

It is extremely penetrating and lasting, and cannot be distinguished from that of an ordinary musk; it is quickly absorbed by water and retained for several weeks even after the gelatine has been removed.

To my knowledge this is the first instance that has been recorded where any peculiar odour has been detected in eggs or their envelopes. It may possibly serve as an antiseptic to prevent the too speedy putrefaction of the gelatine, or perhaps to attract parasites and infusoria, which latter furnish food for the young larvæ.

There is no reasonable doubt as to the identity of the species that laid the eggs. Both males and females of this insect, which Dr. Hargen, of Cambridge, Mass., has kindly identified for me as *Neophalax concinnus*, were observed in great numbers just before twilight flying about over the surface of the water; although watched very carefully they were not observed to oviposit. The eggs are probably laid after dark. Gravid females were found containing eggs and a gelatinous secretion identical in odour and appearance to those found in the water.

Eggs in different stages of development vary considerably in size and shape. They are spherical during the formation of the embryonic membrane as well as after the revolution of the embryo; in all other stages previous to revolution they are oval.

As development proceeds the eggs increase in size to a very marked degree, and by the time they are ready to be hatched the diameter has increased by one fourth or one fifth of its original size. Rathke was the first to call attention to this peculiarity; he observed it in the case of "*Gryllotalpa*" as well as in Phryganids. I have not only been able to confirm his statements in regard to Phryganids, but also to extend them to one of the Locustina, i. e. *Æcanthus*, and to *Blatta*. In both the latter cases the increase in size could not be due to the appropriation of material from gelatinous envelopes since there are none. In all cases the increase in size of developing eggs is probably due to the conversion of a large amount of

highly concentrated and compact food yolk, containing fats and oils, into a less dense and more irregularly distributed protoplasm by the absorption of water, oxygen, and nitrogen from the surrounding media. Oxydation is necessary for all vital activity, and nitrogen is necessary for the conversion of fatty substances into protoplasm. A certain amount of moisture is necessary for the development of eggs which are not normally deposited in water, for such eggs will not develop in a dry atmosphere.

The eggs when first deposited are distinguished by the whitish colour and hard, compact appearance of the gelatinous matrix, which slowly swells and becomes very transparent through the action of the water and decay.

Zaddach states that the larvæ feed upon the softened gelatine and the numerous Infusoria which it contains at the time of hatching. As far as the gelatine itself is concerned he is probably in error, since the larvæ on rupturing the chorion leave the jelly as quickly as possible. Moreover, a great many of these empty gelatinous masses were found which did not indicate that the larvæ had fed upon them. I do not doubt, however, that the larvæ find a welcome store of food in the various Infusoria which infest the gelatinous matrix at the time of hatching.

The question at once arises to what use can these envelopes be applied. To be sure they hold the eggs together in one mass, but as regards protection from their enemies there is more danger in this way than if they were scattered irregularly about. The eggs are undoubtedly eaten by other animals, and when collected into aggregates all suffer the same fate, which would not be the case if they were isolated.

In all Phryganids, with the exception of the present species, the gelatine serves to fasten the eggs to various objects of more or less stability. The eggs of *Neophalax*, as well as some species of *Chironomus*, are suspended in a jelly which floats or lies freely in the water. In other species of *Chironomus*, according to my own observations, the eggs are fastened to some stick or stone.

We have already seen that the gelatinous envelope swells on contact with water, thus decreasing the specific gravity which tends to hold the eggs more freely suspended in the water and prevents them from being too deeply buried in the mud and slime which would cut off the necessary supply of oxygen.

It is possible that the gelatine may have some differential effect upon the diffusion of gases held in the water, excluding some and hastening the transmission of others. It may also serve as an attraction to Infusoria which feed upon the decaying mass, and thus furnish a ready supply of food for the young larvæ. But it is certain that in the great variety of instances where a similar covering is present in other animals which deposit their eggs in the water, e.g. Molluscs, Amphibia, Reptiles, &c., that it must have some other function than the ones just mentioned. For in all the cases mentioned above where we have pointed out a real or supposed advantage accruing to one species or another, it is easily seen that to other closely related forms, and were nearly the same conditions were present, there would be no advantage whatever. No one of these advantages is of such general importance in all the different cases where we find the jelly present as to warrant us in supposing that it was primarily present for that purpose, or, at least, that it had its principal function therein.

There is, however, still another function to which this envelope may be applied, and indeed it is one of far greater and more general application than any as yet suggested. It is, namely, that the gelatinous envelopes of certain eggs which are deposited in the water have their chief function in protecting the eggs against the parasitic growth of fungi and Infusoria. I have observed a great many times that when the gelatine which envelopes the eggs of *Bufo* or *Rana* has been ruptured and exposed the eggs to a more direct contact with the water, that they are very quickly covered and destroyed by fungi. It might well be claimed that the same agent which ruptured the envelope might have killed the embryos, and that of course putrefaction and parasitic growths would follow. But this objection can be easily met by the fact that not only the eggs

nearest the rupture begin to decay, but also five or six, or even more, which lie at varying distances from the rupture, and show more or less decay and parasitic growth in proportion as they are near to or far from the break in the gelatine. It is not difficult to find molluscs' eggs (*Lymnaeus*) where a similar rupture has occurred, and the subsequent growth of fungi has taken place.

In the case of *Æcanthus*, which deposits its eggs in holes bored in the small branches of various shrubs, if the eggs are removed artificially and kept in a damp place they become quickly covered with such a thick growth of fungi as to prevent further development by shutting off the necessary supply of oxygen.

II. PREPARATION OF THE EGGS.

Heat alone was found to be the simplest and best means of hardening the eggs and larvæ, which are placed alive in cold water that is very gradually raised to about 60° R.; the proper time to stop the rise in temperature is when they become hard and white. They are allowed to stand till cool, and then removed to 20 per cent. alcohol, which is increased by 10 per cent. once or twice a day until it has reached the required strength, or about 96 per cent. This method has been used with the best success for insect larvæ whose flexible, but impervious, chitinous skins will not allow reagents to penetrate when used in the ordinary way. The great advantage of this method is that it coagulates the tissues without contracting them; the expansion of the air contained in the body prevents the shrivelling of the larva which results when it is treated with cold reagents.

The eggs and larvæ were also heated in Kleinenberg's picro-sulphuric acid solution, one third the normal strength, but with far less satisfactory results.

Since writing the above I have preserved a considerable number of insects' eggs. When picro-sulphuric acid was used and made to penetrate the chorion by means of heat, it was

found almost impossible to remove it again. Eggs of a species of water-beetle—which I believe to be *Hydrophilus*—that were preserved in this way have now been in strong alcohol (96 per cent.) for six months, and they still have a very perceptible yellow colour, due to the picric acid which still remains, and makes it very difficult to stain them with hæmatoxylin. A number of experiments have also been made in the use of corrosive sublimate, but with very unsatisfactory results; the eggs were rendered very brittle, and good staining, which is the chief difficulty in the study of insect eggs, was made impossible. Insect eggs seem to have the power to withstand in a great degree the penetration of staining fluids, which is probably due to the compact tissues, rendered much more so by the use of sublimate, since it causes a considerable shrinkage. At all events, if the eggs are killed by a prolonged and gentle heat, and then hardened in alcohol, no difficulty will be found in staining them, and the histological characters are preserved in as much perfection as by any other method at present at our command.

Bobretzky was the first to use heat in the preparation of eggs for sectioning, but he used it principally as a means to increase the penetration of the chromic acid, a reagent which adds materially to the difficulty of staining them.

The small size of the eggs made it impracticable to remove the chorion, the presence of which proved a serious obstacle to the penetration of staining fluids. Only alcoholic stains would penetrate at all, and of these Kleinenberg's hæmatoxylin gave by far the best results. Partial and varied success was obtained from saffanine dissolved in 90 per cent. alcohol, and Grenacher's alcoholic borax carmine. Saffanine gave better results than the carmine, since it never failed to stain, whereas the latter did.¹

For the early stages of the egg a light red stain like cochineal

¹ Later experiments, with a 70 per cent. solution of cochineal, have given very good results indeed; and in cases where it is difficult for the fluid to penetrate, this stain produces the required result far better and more rapidly than all others.

or saffanine is the best, since the sections can be cut a little thicker, and still be transparent enough to show the cell structure. This proved of great advantage, since in the early stages the sections, if cut too thin, were liable to crumble on account of the abundance of yolk. When the eggs were stained with hæmatoxylin they were allowed to remain in the staining fluid for five or six days, and were then decolourised very gradually in an extremely weak solution of alcohol and hydrochloric acid—one drop of strong acid to about twenty grammes of alcohol—in which they are allowed to remain several days, and then transferred to pure alcohol, which is changed once or twice until the object has regained its violet colour. Out of a cluster containing about one hundred and twenty-five eggs, only one half or even less could be properly stained. Some were stained only on the outside, although appearing as dark as the rest. By the process of decolourising, such eggs were easily detected and picked out. Some eggs stained more rapidly than others; by leaving them five or six days in the fluid, such as could be at all affected were over-stained. When placed in the solution of hydrochloric acid, all were decolourised alike. The eggs were next transferred successively into absolute alcohol, clove oil, and paraffine, remaining several hours in each.

In treating subsequently the eggs of "Blatta" some changes were made which proved of great advantage. Benzole was used in the place of clove oil; it rendered the eggs transparent almost instantly, and since it is more fluid than clove oil, there is much less danger of the embryo being shrivelled, while at the same time the yolk is not made so hard and brittle. After remaining in benzole for half an hour, the eggs are changed to a saturated solution of benzole and paraffine, which, when allowed to evaporate slowly, gives a perfectly transparent and tolerably hard paraffine. They are next transferred to melted paraffine, which at once replaces the benzole expelled by the heat. Thus there is a great saving of time, since clove oil is not thoroughly replaced by paraffine in less than five or six hours, while with benzole one half an hour is

sufficient. When it was necessary to cut sections of the larvæ, it was found of the utmost importance to transfer them from absolute alcohol to benzole, and then into a solution of benzole and paraffine in the manner described above. The larvæ invariably collapse when placed in clove oil or turpentine. When they are to be mounted in toto the same principal can be applied by mounting them in balsam dissolved in benzol; the solution which can be used thin at first may then be allowed to evaporate slowly until the balsam is of the required density.

On account of the small size of the eggs it was necessary to cut very thin sections. For this purpose the hardest paraffine was required; the melting point of that used was fifty-eight degrees centigrade (58° C.). To get good sections the embedding material must be perfectly clean, hard, and free from bubbles; these conditions can be obtained only by the utmost care in manipulation and in the arrangement of details. Certain advantages are gained by attention to the form of the blocks of paraffine in which the objects are embedded; they should be in the shape of a triangle, the base, which is the shortest side, being just wide enough to hold the object, and the altitude about five or six times the length of the base. The apex of the triangle is directed towards the edge of the knife, which, if placed at an angle of 10° or 12° to the direction in which it slides, will reach the apex of the triangle first, and cut a closely rolled shaving of paraffine. The section of the object, since it lies in the last portion of the section cut, will occupy the outside roll; it is obvious that, if the triangle was long enough, the roll of paraffine would be so large that a section of the egg would not cover more than one half the circumference. The whole roll may now be placed on the slide, with the section of the object down. When the slide is warmed, the melted paraffine spreads the sections, and thus the trouble of unrolling is avoided.

If in cutting the sections the edge of the knife is placed at right angles to the direction of motion, the sections will not roll, but at the same time they cannot be cut so thin.

Occasionally it is necessary to partly unroll the sections. In this case care should be taken not to use too much clove oil in softening the shellac, or the sections will not adhere firmly enough to allow them to be unrolled. The shellac is also more adhesive if put on the slide just before using. With attention to these details, a roll of paraffine will adhere firmly to the slide, and then may be easily unrolled by means of a fine-pointed needle. When all the sections have thus been placed in rows upon the slide, it is warmed, melting the paraffine, and allowing the sections to lie smoothly on the surface. A very important point is not to warm the slide too quickly, for if there are any sections which do not lie directly on the surface, the paraffine which holds them together will melt and let them fall to pieces before they have had a chance to fasten themselves to the shellac. Hence the slide should be warmed very gradually, and the sections made so flexible that they fall of their own weight into place without the paraffine being actually melted. Moreover, if the slides are heated too much, the shellac and paraffine will unite to form a whitish film, which often destroys the value of the preparation. After cooling the paraffine is dissolved in turpentine or benzole, and the sections mounted in Canada balsam. By this method one hundred sections have been cut from an egg which was one hundredth of an inch in diameter, or, in other words, a nearly complete series of sections which were a little more than one four hundredth of a millimeter thick.

III. HISTORICAL.

In spite of the innumerable works which have been published concerning the anatomy and habits of insects in general, very little notice has been taken of their embryology. It is remarkable that while our present insufficient knowledge has pointed out such a diversity in the development of closely related forms, that so little attention should have been given to the development of the different groups. The Phryganids, however, have received their proportionate share of attention, since already we have six more or less fragmentary observations concerning

their embryology. One of the earliest references was published by Rathke in Müller's 'Arch.,' 1844. In a footnote (p. 27) he called attention to the increase in the size of the eggs during development. In the posthumous papers of the same author, published by Hagen in the 'Stettiner Entomological Zeitung' for 1861, mention is made of an apparatus for rupturing the egg membranes at the time of hatching. Independently of Zaddach, and, indeed, previous to him, Rathke had also observed the revolution of the Phryganid embryo in the egg, although his notes were not published until several years after the appearance of Zaddach's work. He also described the general external appearance of the eggs, and their occurrence on the under side of the leaves of water plants, *Hydrocaris morsus ranei*, *Nymphæ*, and *Polomagaton*, &c., as well as their arrangement in concentric circles one layer deep. The eggs, one hundred or more in a single cluster, had two membranes, a thick outer chorion, slightly tessellated, and an inner very thin vitelline membrane; the latter was present when the eggs was first laid, but in subsequent stages he was not quite so positive of its existence.

A faint "Keimhaut" was formed containing nuclei. Development was completed in three weeks. Four long and spirally-coiled Malpighian vessels arose behind the stomach. No gills were observed when the larva was first hatched, but thread-like flexible structures appear later which he supposed to be gills. There was no trace of abdominal appendages.

By far the most important work on the development of "Phryganids" that has yet appeared is the memoir published by Zaddach in 1854. I will therefore give an outline of the more important conclusions reached by this author. The eggs are found in single layered masses on the under side of the leaves of water plants, and are from .009 inch to .011 of an inch in diameter. There are two membranes, an outer, smooth, transparent chorion, and an inner, thin, vitelline membrane. Shortly after oviposition clear spots appear on the surface of the yolk, which gradually increase in number and finally become confluent, forming a transparent outer layer; this, in

his opinion, is a viscid fluid formed by the dissolution of the yolk ; when it has covered the latter uniformly there appears in it isolated white spots—the germ nuclei. These increase in number, and finally cover the yolk with a continuous cell layer. It is claimed here, in accordance with the ideas prevalent at that time, that the nuclei arise first, and that later a cell membrane is formed around them. As soon as the blastoderm is formed its cells are concentrated towards one side, thus leaving the opposite side of the yolk covered by a thin layer, which subsequently ruptures and allows the yolk to come in contact with the vitelline membrane. The germinal band which results from these changes occupies two thirds or three fourths of the circumference of the egg.

The second period, including the metamorphoses of the germinal band and the development of the appendages, begins with two important changes in the former. 1. Its separation into two layers. 2. The formation of two germinal folds or “Keimwülste” in the inner layer. The two layers are formed by delamination in the head, and gradually extend to the posterior end of the body. During the formation of these two layers the germinal band not only becomes much thinner in the midlongitudinal line, but on the side towards the yolk a deep furrow is formed by which the germinal band is partially divided into two lateral ridges, the “Keimwülste” thus at an early period indicating the bilateral type and furnishing foundation for the development of the segments and appendages. The inner layer, after giving origin to the nervous system and appendages, forms the muscles of the body walls, and hence is called the “Muskelblatt.” The outer layer forms the external covering of the sides and dorsum, the subsequently hardened skin of the embryo, and hence is called the “Hautblatt.”¹

The procephalic lobes are formed by the thickening of a pair of lateral outgrowths from the “Hautblatt” in the head region. After their formation and that of the oral invagination the “Hautblatt” ruptures in the middle line throughout nearly the

¹ The “Hautblatt,” without doubt, corresponds to the “amnion” and serosa together.

whole length of the original germinal bands. In some cases the rupture is continuous along a transverse line in the head region, and thus a kind of frontal lappet of the "Hautblatt" is left on that portion of the yolk which limits the anterior end of the head. This lappet encloses the so-called head cavity which lies between the procephalic lobes; when, however, in such cases the posterior end of the embryo has grown long enough to reach the head, it fuses with this lappet and thus obliterates the difference between this and the more normally produced condition.

The appendages are evidently developed from the germinal ridges, and push the edges of the ruptured "Hautblatt" away from the middle line, which now exhibits renewed activity along its lateral margin where it remains continuous with the "Muskelblatt." This activity is manifest in a thickening of the "Hautblatt," due to rapid cell-formation which progresses from the lateral to the median edges, and also in the upward growth of this layer which thus envelops the yolk.

The mandibles first arise and then appear simultaneously behind them the five following segments. Although the antennæ appear to develop from the procephalic lobes they are believed to arise from a distinct segment of their own in front of the mandibles, and to correspond to the second pair of antennæ in Crustacea, whereas the antennæ of the adult insect are claimed to be homologous with the first pair of Crustacean antennæ.

The primitive germ band includes hardly more than the future head and thorax. It increases in length by means of new cell formations, which result in the production of an abdomen by the elongation of which the end of the tail comes to lie next to the mandibles. The abdomen consists of ten segments.

The author concludes from the way in which the anal stylets are developed that they are true appendages.

The third period extends from the time of revolution until that of hatching. Four important changes occur in this stage. 1. The conversion of the loose cells into a compact granular tissue from which are formed the different organs. 2. The

closing of the "Scheitel Platte" to form the brain case and the growth of the "Hautblatt" around the yolk, by means of which the body of the embryo becomes enclosed. 3. The gradual contraction of the germinal folds, by means of which the embryo, hitherto encircling the yolk, now comes to lie with its back turned outward, and, 4, the formation of the mesenteron and all other vegetative organs.

Just before revolution the "vitelline membrane" ruptures and collects on the exposed portion of the yolk between the head and tail where it forms a kind of rosette. The author is in doubt whether it forms a part of the dorsal wall or is absorbed by the yolk. The time of the rupture of the vitelline membrane varies, and may even be deferred until after the revolution of the embryo. Sometimes the membrane is absent altogether. The under lip is a ridge-like elevation, which is formed from the mandibular and two maxillary segments with the second maxillæ as a palpus.

After revolution, which is caused by the contraction of the ventral side of the embryo, the latter becomes spirally rolled in the egg, and retains this position until hatching.

The wall of the stomach is formed first on the ventral side and thence grows dorsally.

The eye first appears as six pigment spots. In some cases the lenses to the compound eye could be distinguished.

A sharp-pointed organ found on the forehead serves to rupture the egg membranes. The development covered a period of from eighteen to twenty days.

Previous to Zaddach, Jeder had made some very scanty observations on the nervous system and the eye.

In 1871 Kowalevsky confirmed Zaddach's observations on the membranes, which, after rupturing, form a kind of rosette on the exposed surface of the yolk between the head and tail of the embryo. These observations were made by Wagner, and communicated by him to Kowalevsky.

Brandt, in 1878, referring to the formation of a blastoderm in Phryganids described a clear outer layer in which nuclei subsequently appeared. The origin of the cells was not

observed, but in harmony with his theory he believes that they are direct descendants of the germinative vesicle.

Dohrn, in 1876, also noted the rupture of the membranes and the formation of a dorsal organ. He says that it finally disappears, but does not state in what manner.

IV. DESCRIPTIVE.

Stage 1.—In this stage will be included an account of the egg from the time it is oviposited until the formation of blastoderm.

The youngest eggs observed were usually ovate in outline, and often more or less irregular from mutual pressure. The viscid, greenish yellow yolk completely fills the chorion and contains two kinds of rounded elements; those of one kind are albuminoid in nature, staining deeply in all the common reagents; those of the second kind are colourless, much more refractive than the first, and exceedingly variable in size. They are dissolved in the processes preceding imbedding, so that in sections their former existence is indicated only by the presence of circular cavities in the yolk; from this it is to be inferred that they are oil globules.

Within ten or or twelve hours after oviposition, the time varying with the temperature, a clear space makes its appearance at the surface of the egg, and gradually increases until it has attained the breadth of the future blastoderm. In this layer, which has been called the "blastema,"¹ the protoplasm has, under ordinary conditions, a very homogeneous appearance, with occasionally lighter, less refractive spots which appear like vacuoles, but in which, when observed more closely and under slight pressure of a cover-glass, or especially when treated with a very little acetic acid, faintly marked nuclei

¹ There is not the slightest doubt that in many insects a structure precedes the true blastoderm, presenting many well-defined characteristics which distinguish it from the blastoderm; hence it requires a special name. I can see no objection to retaining the name "blastodermic blastema," or simply "blastema," originally proposed by Weisman, even though his view that the nuclei of the blastoderm arise in this layer spontaneously is incorrect.

make their appearance in greater or less numbers, according to the more or less advanced stage of the blastema.

From the study of the living egg one might readily infer that the nuclei arise as free formations in the protoplasm, since in no case could they be observed to ascend from the depths of the yolk into the "blastema;" and, indeed, Weisman claimed this to be the case in the *Diptera*, although later he withdrew his statement. Strictly defined, and in the sense that we use the term, the "blastema" is a thin, nucleated layer of protoplasm covering the whole outer surface of the yolk, and not divided into distinct cells. It may be remarked here, that it is not impossible, or even improbable, that a "blastema" may occur in some instances without nuclei, although at present this has not been observed to occur. How such a condition could be brought about will appear later.

The true structure, however, of the "blastema" can only be learned by the study of carefully prepared sections, and we shall then be convinced that the above definition is correct. Such sections through the long axis of the egg in the early stages of the "blastema" are represented in Pl. XXXVIb, figs. 1, 2, 3. Here one may observe: (1) spherical masses of yolk more or less modified by contact with each other; (2) yolk masses whose outlines are so modified as to enclose circular cavities; (3) amœboid cells, each containing a large and sharply stained nucleus; (4) an irregular network of protoplasm whose amœboid filaments are usually connected with the cells just described; and (5) a thin layer of protoplasm at the periphery, containing a few nuclei, and continuous with the protoplasmic network in the interior of the egg. Sections of a stage slightly later than the one just described show (fig. 2) that the blastemic layer has increased in thickness, and is supplied with a considerable number of regularly arranged nuclei. Both the protoplasmic network and the cells observed in the previous stage have disappeared from the yolk. In the next stage observed (fig. 3) the protoplasmic layer has become more sharply defined, the nuclei being more numerous and regularly arranged than in the previous stage. The surface of

the protoplasm has an undulating outline, showing the first indications of division of the syncytium into distinct cells. At this period also no nuclei are to be seen in the yolk. The explanation of these facts is very simple. The nuclei first seen in the yolk have arisen by the division of the germinative vesicle, and together with the protoplasm scattered through the yolk are migrating towards the surface. In the later stages it will be observed that all the germ cells have reached the surface, forming a syncytium, the blastema, which speedily develops into the blastoderm.

There is considerable variation in the relative amount of protoplasm and the number of nuclei which may be found at the surface at a given time. Very often the protoplasmic layer would attain a thickness equal to that of the future blastoderm, and yet contain but a very few scattered nuclei; while, on the other hand a large number of nuclei could be seen at the surface, where there was hardly enough protoplasm to form a continuous layer. Hence it is not only possible, but also probable, that a blastema might in some cases occur in which there were no nuclei. For if in one species, as in *Neophalax*, we can have a blastema containing a very few or a great many nuclei, it is not improbable that in other insects the variation may be carried a step farther and produce a layer of protoplasm in which there are no nuclei. Such a modification would be of extremely slight morphological value, since it is a condition which is dependent on the fact that sometimes the protoplasm reaches the surface first, and sometimes the nuclei. We have, however, seen that such variations can actually exist in one species. Still, the fact remains to be proved by means of sections that a blastema actually does exist without nuclei.

The yolk¹ presents two varieties; in one the globules are perfectly homogeneous, and stain deeply; in the other they

¹ The greater part of this section on the structure of the yolk applies chiefly to *Blatta*, on which the most complete observations were made. From what was seen in Phryganids, I should think that the same description would apply to that group also. In Phryganids the yolk is not so compact, and the amount of protoplasm is proportionately much larger.

stain less deeply, and are composed of a loose, apparently granular mass, which, however, by closer inspection, reveals the presence of so many irregular vacuoles as to reduce the yolk substance to a fine network. Such yolk masses are most abundant near the surface. They are probably merely varieties of the first kind, brought about by its gradual absorption to supply food for the rapidly developing germ cells. It is an important characteristic of the yolk that it is not transformed into protoplasm simply by the solution of its outer boundary, as a small block of ice would be dissolved in warm water; on the other hand, the yolk balls do not decrease perceptibly in size, but simply become less dense, and finally are changed into a more or less fine network of deutoplasm, the walls of the yolk ball still retaining its sharply defined outline. It is possible that these cavities in the deutoplasm may have been filled with an oily or fatty substance, which had been dissolved by the reagents used in the preparation of the egg. That a great deal of soluble fatty substance is present in the yolk from the first we have already seen; but from the configuration of the solid yolk around spaces once filled with fat we can see that a great deal of the substance in question was formed of isolated globules. Since in the very early stages of development the yolk has that homogeneous appearance first described, and the granular yolk only appears after the germinal cells have begun to increase rapidly, the only explanation available is that this transformation of the yolk is brought about either by the gradual absorption of the yolk at many different points in each globule, or the yolk may be first gradually transformed into small fat globules which are then converted into protoplasm, and absorbed by the amœboid cells. If the eggs had been treated with alcohol and benzole, this fatty substance would have been dissolved, producing the net-like or granular yolk balls just described. It is indeed not easy to decide by which of these processes the yolk is absorbed, since we have no facts at hand which point more plainly towards one conclusion than another.

If the yolk was decomposed by direct contact with the

cell protoplasm, we would expect to find a close coincidence existing between the distribution of the germ-cells and the granular yolk. This is, however, not the case, for if we examine sections of the eggs of *Blatta* cut through the longitudinal dorso-ventral plane we shall find, when there are only a few cells in the centre of the egg, that the deutoplasm has already become granular at the periphery; later, when the cells are distributed regularly throughout the egg, that a very thick layer of granular deutoplasm has been formed on the ventral side directly under the place of the future ventral plate. Hence it would appear that the yolk transformation goes on independently of the germ cells. The fact that the granular yolk is found for the most part at the periphery indicates that the decomposition was produced by a kind of breathing process. This supposition is rendered improbable by the fact that the ventral surface of the egg, which has the greatest amount of granular yolk, has the least air, being turned towards the centre of the cocoon in which the eggs are laid. Since the transformation of the yolk is not governed entirely by the supply of air, although we cannot deny that a certain amount is a necessary condition, and also, since it is not produced by the direct action of the living germ-cells, we must look to some other agent for the conditions requisite for bringing about such a transformation. Since then, the yolk is metamorphosed on the ventral side, anticipating, as it were, the advent of the germ-band, and supplying beforehand the necessary amount of ready food for the subsequent rapid development of cells at this point, it may be influenced by the same conditions which determine the subsequent location of the germinal band at that place.

Stage 2.—The second stage is of comparatively short duration, and is marked by only two important features—the formation of the blastoderm and its differentiation into the ventral plate.

The blastoderm is formed by the simultaneous aggregation of a definite mass of protoplasm around each nucleus of the blastema. These masses quickly acquire cell walls, thus forming

the first true cells of the embryo, for hitherto we have had a "syncytium." By mutual pressure the outer faces of the cells become highly convex. The nuclei are large, and each contains a small, sharply-stained nucleolus. When seen from the surface they appear as represented in Pl. XXXVIA, fig. 1; in section as in Pl. XXXVIB, fig. 4.

The blastoderm at first surrounds the yolk as a single layer of cells of uniform thickness, which soon become long and columnar at one pole of the egg, and correspondingly thin and epithelial-like at the opposite pole (Pl. XXXVIB, fig. 5). The thin portion of the blastoderm becomes the "serosa," and the thickened part is the ventral plate. Zaddach states that the blastoderm ruptures at the dorsal pole, where it becomes so thin. This, however, is a mistake, as a long series of sections at this stage, and even much later, shows the complete continuity of the original blastoderm at all points of the egg.

Stage III.—The period covered by this stage extends from the time of the formation of the ventral plate up to the completion of the embryonic membranes. It includes an account of the gastrulation, the origin of the endoderm, and the mesoderm, as well as the formation of the amnion and serosa.

The embryonic membranes are first seen as faint elevations of the "ventral plate," which in optical section appear like a thick layer of columnar cells (Pl. XXXVIA, figs. 3 and 4). In actual section they appear like simple folds of the blastoderm (Pl. XXXVIB, figs. 8 and 9), which are raised on all sides of the embryonic area, and gradually increase in extent until they finally meet nearly over the middle of the ventral plate. For the sake of convenience in description one may recognise four separate folds—a head fold, tail fold, and two lateral folds—although, as we have already indicated, they form a continuous, somewhat circular, elevation. Pl. XXXVIB, fig. 8, represents a longitudinal section through the ventral plate, showing the head fold at *am''* and the tail fold at *am'*; the latter arises first, and grows more rapidly, often covering more than two thirds of the ventral plate before the lateral folds meet in the central line. This condition is not easy to observe in the living egg, but

a very fair idea of it can be obtained by comparing Pl. XXXVIA figs. 3 and 4.

The ventral plate has a characteristic dome-like shape, so that its outline is strongly arched, both in cross and longitudinal sections.

At this stage the ventral plate is not much longer than it is broad. The cells composing it are columnar, with well-defined, rounded outer ends; the inner ends, on the contrary, are faintly marked, and send delicate, pseudopodial filaments into the yolk. The protoplasm is very faintly granular; it is also vacuolated, and especially in the vicinity of the nuclei. Bobretzky¹ speaks of vacuoles in the endoderm cells of *Oniscus*, which possibly may be of the same nature.

During this stage of development certain nuclei present very striking and characteristic appearances. Although most abundant during this stage they may be observed to occur with varying frequency up to the time of the revolution of the embryo. The most noticeable but less frequent form is that which has a very deeply-stained margin, and contains a highly-refractive, non-stainable substance, in the centre of which may occasionally be observed fine granules, scattered about irregularly, or so concentrated as to form a kind of central axis (Pl. XXXVIc, fig. 33). Another variety is seen in the nuclei represented in Pl. XXXVIc, fig. 35): they are sharply limited, and contain very darkly stained filaments, which at first sight appear like granules, but by careful focusing with a highly-magnifying lens one can distinguish a continuous network of fibres, which in optical section look like granules; they are in marked contrast with the highly refractive and non-stainable substance which constitute the remainder of the nucleus.² A third variety is seen in the great majority of cells which constitute the embryo; they stain deeply and uni-

¹ N. Bobretzky, "Zur Embryologie des *Oniscus murarius*," 'Zeit. für wiss. Zool.,' Bd. xxiv, 1874.

² Compare H. Flemming, 'Zellsubstan, Kern und Zelltheilung,' Leipzig, 1882.

formly, aside from the minute, faint granules, and the small, darkly-stained nucleolus.

A spindle metamorphosis was not observed in the embryo, although the nuclei often contained two nucleoli, and were probably undergoing rapid division. The small size of the nuclei, however, rendered them unfavorable for the study of such phenomena.

These different kinds of nuclei are distributed irregularly throughout the ventral plate, and are so constant in their characters that it is difficult to find stages which might be considered transitional. The first two varieties possibly represent stages preliminary to division. It should be noticed that the ordinary nucleus in a state of division presents no other peculiarity than the presence of two nucleoli.

It will be seen on examining Pl. XXXVIB, figs. 8, 9, and 10, that the nuclei of the ventral plate are uniformly located at the inner or deep ends of the cells, while those of the amnion are at the outer ends, thus maintaining in both layers the same morphological position. From this we can infer that the amnion is simply a fold of the thickened ventral plate, and the serosa merely the slightly modified blastoderm covering the remainder of the egg, and which gradually extends over the ventral plate in a fold, whose lips finally meet and fuse, thus producing two completely closed sacs.

It can be seen, in Pl. XXXVIB, fig. 8, when the folds are first forming, that the inner limb (*am*) has the same thickness as the ventral plate itself, and also that the nuclei have the same morphological position, while, on the other hand, the outer limb (*sc*) is very thin, being simply a continuation of the blastoderm. These relations are still perceptible in much later stages (figs. 9 and 10), even when the membranes are completely formed (fig. 10). Thus, according to this view, the real edge of the ventral plate is not where the amnion joins the ventral plate, but where the amnion is continuous with the serosa.

At present I cannot claim any special morphological value for these distinctions, since all the parts in question are directly

descended from the blastoderm ; neither is it possible as yet to say how far such distinctions hold good in other forms. Judging, however, from Kowalevsky's¹ figures I should think that the amnion was a derivative of the ventral plate. It is the same in *Porthesia* figured by Bobretzky,² although the difference is not so well marked here as in *Hydrophilus*.

We have already seen that the fusion of the amniotic folds with each other produces two completely closed sacs, one within the other (Pl. XXXVIb, figs. 10—13). The outer sac formed by the serosa, which is not fully represented in the figures, completely encloses the yolk. The second sac, the amnion, is also completely closed, its dorsal wall being much thickened to form what we shall presently describe as the "germinal band."

We have seen that the ventral plate in the restricted sense in which we use the term is simply an oval thickening of the blastoderm covering the ventral side of the egg. During, however, the formation of the amnion and serosa this thickened area takes a more definite shape, increasing in length and decreasing in breadth, thus giving rise to a slipper-shaped thickening, which we shall now call the "germinal band." The head of the future embryo arises from the "heel" of this band, which at this place expands into two plates, one on each side, in order to form the procephalic lobes. Zaddach's statement that they arise as thickenings of the "Hautblatt" is erroneous ; for, as far as I am able to make out, he confounded the amnion, or possibly both amnion and serosa, with the ectoderm.

Up to this time the germinal band consists simply of a single layer of similar cells ; it, however, continues to increase in length, until at the end of this period it extends over two thirds of the circumference of the egg.

Meantime, however, there have occurred peculiar changes affecting the apparent localisation of the ventral plate and germinal band. The thickening of the blastoderm occurs at one

¹ A. Kowalevsky, "Embryologische Studium an Würmern und Arthropoden," 'Men. Ac. imp. St. Petersburg,' Ser. vii, vol. xvi, 1871.

² Bobretzky, "Ueber der Bildung des Blastoderms u. Keimblätter bei den Insecten," 'Zeit. für wiss. Zool.,' 1878, vol. xxxi, pp. 195—215.

pole of the egg, and may be said to lie parallel with its short axis (Pl. XXXVI_B, fig. 5, and Pl. XXXVI_A, fig. 2). During the formation of the embryonic membranes the egg becomes spherical; and still later, just before the appendages have appeared, it again assumes an oval form, the ventral plate now, however, extending in the direction of the long axis of the egg.

These changes are brought about by an increase in the length of the ventral plate, and its tendency to lie straight in the egg. This is rendered possible by the flexibility of the chorion, which yields to such an extent as to readily allow the transformation of the short diameter into the long one. When the ventral plate has attained about one half its ultimate length the egg assumes a circular form, owing to the equilibrium existing between the extensive force of the germ band and the tendency of the egg to retain its oval shape. This circular form, however, is retained but a comparatively short time, for in about twenty-four hours the germinal band has so increased in length as to convert what was the short axis into the long one (compare Pl. XXXVI_A, figs. 4, 5, and 6); here the egg is seen from the dorsal side, and simply shows the two ends of the germinal band, which has already begun to encroach upon the dorsal surface of the egg.

During the formation of the embryonic membranes there may be observed a median longitudinal infolding of the germinal band, which commences in the posterior end of the embryo and gradually extends towards the head, almost reaching the point where the mouth appears later; in optical section it has the appearance shown in Pl. XXXVI_A, fig. 5. It will be seen in the next stage that this fold entirely disappears (Pl. XXXVI_B, fig. 11), and is not to be confounded with a median fold in the same place that appears later, and which initiates the formation of the nervous system (Pl. XXXVI_B, fig. 12). The first furrow gives rise to a part of the endoderm and all of the mesoderm, hence we will call it the "gastrula."¹ The

¹ Objection has been made to the use of the term "gastrula" in this connection, on the ground that the invaginated cells do not give rise to the endoderm. This objection has in the present instance been met by showing that

second furrow gives rise to a part of the nervous system, and hereafter we shall call it the "neural furrow."

The gastrula then is quite open at first, but its lips gradually close, so that the only trace of the opening left is that shown by the arrangement of the invaginated cells and their nuclei (Pl. XXXVIB, fig. 10). The closing of the gastrula mouth begins at the posterior end of the body, and gradually extends towards the head end. In no case do the infolded cells form a closed tube. In two or three hours the furrow has entirely disappeared.

The cells which are thus permanently cut off from a direct share in forming the outer wall of the body constitute the beginning of the mesoderm. Although the boundaries of these cells are no longer sharply defined, the position of their nuclei shows clearly the relation which they sustain to the rest of the ventral plate, and is suggestive of the stages which have immediately preceded. As seen in Plate XXXVIB, fig. 10, these nuclei are already arranged in two rows approximately parallel with the nuclei of the neighbouring ectoderm. With the depression of the dome-shaped ventral plate which occurs at this period, the mesoderm is changed into a single layer of cells covering the under surface of the ventral plate (Pl. XXXVIB, fig. 11). This movement, however, may not necessarily imply any marked amœboid properties for the cells at this period; they have increased in numbers, and the shifting incident upon cell division has played an important part in the changes of position that have been observed.

Zaddach's description of the splitting of the ventral plate into a "Hautblatt" and "Muskelblatt" can in no way be compared to the formation of the mesoderm as described above.

at least a part of the endoderm does arise from invaginated cells. But even if this was not so, I cannot see that it would influence our use of the term in this case. For it seems to me that an inherited tendency to produce an invagination, the sole object of which is to cause a differentiation into germ layers, however successful or unsuccessful the attempt may be, should receive the name that was originally applied to the more simple condition, always holding in mind, however, the fact that it is a condition modified by various agents, the nature of which may or may not be known.

What he described as the "Hautblatt" was probably the amnion and serosa combined, and the "Muskelblatt" the ventral plate. The same author has figured an infolding similar to that described as the "gastrula," but which, in his opinion, is simply the secondary result of the formation of two lateral thickenings or germinal ridges out of which the segments and appendages of the body are formed. He states that the ridges result from a lateral contraction, which causes a furrow to appear on the inner surface of the ventral plate, and not by an infolding of the outer surface, as shown in fig. 9, Pl. XXXVIb. It is difficult to decide which of these two infoldings Zaddach had under observation when he wrote his description, but judging from the fact that he ascribed the origin of the nervous system to the germinal ridges on each side of the furrow, I think it probable that what he saw was simply the neural furrow.

The endoderm arises from any point in the blastoderm by DELAMINATION, and this process may continue even after the blastoderm has been converted into the ventral plate.

It will be remembered that no cells were left in the yolk after the formation of the blastoderm. By examining sections of eggs during the formation of the embryonic membranes, amœboid cells, containing large granular nuclei, can be seen in the yolk both in the region of the ventral plate and in that of the serosa; in the latter one may occasionally find cells projecting into the yolk and sending out ray-like arms of protoplasm; very often two cells may be seen close together, one in the serosa, the other just inside the yolk and connected with each other by protoplasmic filaments. Other more or less similar cells can be seen in the immediate vicinity of the ventral plate, especially along the median longitudinal line. Admitting the accuracy of the observation in regard to the entire disappearance of the nuclei from the yolk in the blastoderm stage, there are thus only two possible interpretations for the present condition; either these yolk cells rise autogenously in the yolk and are migrating towards the periphery, or certain cells of the serosa and ventral

plate have undergone division, and one of the products of that division having assumed amœboid characters, is now in the process of freeing itself from the blastoderm preparatory to migrating into the yolk. There can be no doubt as to which of these explanations is correct. There is not the slightest evidence of the free formation of cells within the yolk, and their intimate connection with the serosa and ventral plate points clearly to the cells of these structures as the source of their origin. These so-called yolk or endoderm cells, which later become somewhat modified in size and appearance, are gradually distributed through all parts of the yolk and ultimately form the epithelial lining to the mesenteron.

Stage 4.—This period extends from the completion of the embryonic membranes up to the appearance of the appendages; it is marked by the segmentation of the mesoderm.

The germinal band is now a thick layer of cells, extending over about three fourths of the circumference of the egg, and continuous at its periphery with the amnion, which meantime has become very thin. Surrounding both amnion and germinal band, may be seen the serosa, with its large granular nuclei, from which the amnion can readily be distinguished by the small size of its nuclei. The two cell layers ultimately become very closely united, although never completely fused, since in sections of the embryo just previous to revolution it can readily be seen in many places, especially near the nuclei of the amnion, that they are separate membranes.

The amnion always maintains its continuity with the extremities of the body, so that the amniotic cavity remains completely closed, even up to the time of the rupture of both amnion and serosa. The extreme tenuity of the amnion at this stage is in marked contrast with its appearance when first formed, it being then as thick as the ventral plate.

The invagination which gave rise to the mesoderm and part of the entoderm occurred during the formation of the embryonic membranes, and disappeared when the latter had completely fused with each other, hence no trace of an invagination is to be observed during this stage. It is of great

importance to observe the complete disappearance of the gastrula invagination and the existence of a stage following it where there is no median furrow. The neural invagination, as we shall soon see, is a second and later process, and although it occurs exactly in the place where the gastrula mouth disappeared, it has no connection with it whatever. The definite comprehension of this fact will, I believe, prove quite as important a step towards the comprehension of the phenomena of insect development, as has the similar distinction between primitive groove and medullary furrow in the case of Vertebrates. This distinction between the neural furrow and the gastrula has not heretofore been clearly recognised in insect embryos. Zaddach surely confounded them, and I find no evidence that any observer has called attention to the fact that there are two fundamentally distinct invaginations occurring successively in the middle of the ventral plate.

The mesoderm at the beginning of the present period is unsegmented, and forms a continuous layer of cells beneath the ectoderm; it soon separates along the median longitudinal line, thus forming a pair of lateral mesodermic bands, each of which at the same time divides into segments or somites, which, however, do not contain a lumen or body cavity as described by Hatschuk and Kowalevsky, but are simply formed by the apposition of two cell layers, which later split into the splanchnic and somatic mesoderm.

The ventral plate, as seen in the living egg at this time, shows a gently undulating surface, the external evidence of the segmentation of the body, the depressed areas alternating with the mesoblastic somites. The formation of the external depressions and the segmentation of the mesoderm occur at the same time.

In many places in the mesoblastic somites cells may be seen which present all stages of migration from the mesoderm into the yolk. Some of these mesoderm cells can be distinguished from those surrounding them by having larger and more granular nuclei; others are partly separated from the mesoderm being still united with the latter by a delicate filament of

protoplasm (Pl. XXXVIB, figs. 6 and 7), and still others which have completely separated themselves from the mesoderm and lie in the yolk close to the ventral plate; in general appearance all the cells just described are very similar to each other. Although I am not ready to believe without further evidence that yolk cells arise by proliferation from these segmented portions of mesoderm, yet it is necessary to recognise the fact that the appearances just described could be employed as arguments in favour of such a view. A great many cells similar to those represented in Pl. XXXVIB, fig. 6, were observed in various stages of separation from the mesoderm. In the upper part of fig. 6, Pl. XXXVIB, is represented a cell which is connected with the mesoderm by a very long filament of protoplasm. Such a condition would not be realised in cells approaching the mesoderm, but very likely would be if the cells were separating from the mesoderm; for in the latter case it is very likely that a filament would continue to hold the elements a long time in union. It may be urged, on the other hand, that the absence of any indication of a spindle metamorphosis is, to a certain extent, an objection to the supposition of a proliferation on the part of the mesoderm cells; this objection, however, appears less important when it is remembered that no spindle metamorphosis could be observed in other parts of the embryo where there was undoubtedly rapid cell division.

Stage 5.—The fifth period extends from the segmentation of the mesoderm to the revolution of the embryo in the egg. It is the longest and most complicated period in the development of the insect; at its close nearly all the organs of the body are differentiated.

No important change occurs in the general form of the germinal band up to the time of revolution. The procephalic lobes increase in thickness and at the same time the yolk withdraws from between them, allowing their inner faces to come in contact and unite with each other to form the brain. The germinal band continues to increase in length until its extremities almost touch each other, and even at that late period it is very easy to distinguish in the living egg the points where

each extremity is continuous with the amnion (Pl. XXXVIA, figs. 9 and 10, *am.*¹ and *am.*²). After the appearance of the proctodæum the posterior end of the body becomes bent forward on itself, making the first step towards the revolution of the embryo.

Formation of the Appendages.—Shortly after the growth of the ventral plate has brought the two extremities into close proximity with each other, six pairs of gentle undulations appear simultaneously on the anterior half of the body (Pl. XXXVIA, fig. 8). The three posterior pairs, which ultimately become the thoracic appendages, are somewhat longer than the others. The three anterior pairs become respectively the mandibles, and the first and second maxillæ. As development proceeds each of these appendages assumes a characteristic shape and direction of growth. The mandibles (Pl. XXXVIA, fig. 9) become globose, and lie at right angles to the median plane of the body. The first maxillæ diverge very early from their original rounded form, becoming elongated and pointed at their extremities; they are much larger than either the mandibles or the second maxillæ, and maintain their superiority in size until the embryo hatches. The second maxillæ imitate the first maxillæ in shape and direction of growth.

The three thoracic appendages are very similar to each other in size and outline and in their manner of development. When they are about half grown three pairs of rudimentary appendages may be seen in each of the three anterior abdominal segments (Pl. XXXVIA, fig. 11). The thoracic appendages are at first directed towards the median line of the germinal band, being in marked contrast with the rudimentary jaws, which are directed away from the middle line of the germinal band. No further important changes occur either in the direction of growth or in the shape of any of the appendages until just before revolution, when the second maxillæ fuse to form the under lips and the first maxillæ at the same time turn towards the median ventral line and assume their ultimate arrangement around the mouth-opening. The thoracic appendages become very much distorted just before the rupture of the amnion and serosa (Pl. XXXVIA, fig. 14).

The upper lip is formed by a fold in the germinal band just in front of the mouth; its cavity, which is lined with mesoderm, opens directly into the body cavity (Pl. XXXVIA, figs. 9, 10, 12, 13, 14, and *f. hd.*).

The antennæ appear as knot-like protuberances on the posterior edges of the procephalic lobes (Pl. XXXVIA, fig. 10, &c., *at.*). Here it will be seen that they are very prominent; gradually, however, they become less conspicuous, until in the later stages they are scarcely to be observed at all. When, however, the embryo has emerged from the chorion the antennæ again increase somewhat in size, although still remaining quite small. Zaddach's statement that a second pair, corresponding to the first pair of crustacean antennæ, appears after hatching, the first pair having entirely disappeared, is erroneous.

The nervous system is primarily indicated by a shallow groove, beginning at the anterior end of the body, and extending backwards almost to the tip of the germinal band, terminating at the place where the proctodæum is to be formed. As development proceeds, the cells on each side of the groove divide and give rise to faintly marked nuclei, each of which contains a small deeply stained nucleolus (Pl. XXXVIB, figs. 12 and 13). By the increase in numbers of these cells, a continuous cord is formed on each side of the median furrow. This condition, however, does not last long, for, on the dorsal side of these cords, deeply stained granular nuclei appear (Pl. XXXVIC, fig. 21, *x*), which finally increase to such an extent as to almost take the place of the faintly marked nuclei (Pl. XXXVIC, fig. 23). These granular neural cells at first form a single layer directly under the neural cords. I at first thought them to be transformations of the nuclei above them, but certain indications render it possible that they were formed by division of the ectoderm cells on each side of the neural cords (Pl. XXXVIC, fig. 21, *y*), for in stages slightly earlier than that represented in the figure, these cells were observed only at the point marked *y*, and appeared to grow inwards towards the median line. This point, however, I was not able to determine with certainty.

Soon after their formation the neural cords become transversely divided into ganglia. Meantime the median furrow has increased in depth, forming an invaginated portion of the ectoderm which unites transversely each pair of ganglia. This median portion of the nervous system gradually disappears. Whether it actually forms the cross commissures, as claimed by Hatscheck, is still an open question. While my own observations have not confirmed those of Hatscheck, still, nothing was found that actually militates against his theory.

If we examine longitudinal sections of the embryo after the middle cord has disappeared (Pl. XXXVIc, fig. 37), we shall observe two granular spots or commissures on the dorsal side of each ganglion. These spots increase in extent, and at length fuse with corresponding tracts in the opposite half of the ganglion, thus uniting the lateral halves of each pair of ganglia by two cross commissures (Pl. XXXVIc, figs. 37 and 38). When the embryo is ready to hatch these double commissures disappear; whether they appear again in the adults of this insect I am not able to say. Such commissures, however, are present in the adult crab, and certain insects—*Locusta*—according to Leydig; but whether they are of the same nature as those seen in embryo Phryganids, or whether they are secondary modifications of these embryonic conditions, is a question that we are not in a position as yet to answer. At all events, I have not been able to find a description of such a condition in either insect or crustacean embryos.

Coincident with the ectodermic invagination joining the lateral halves of each ganglion, transverse invaginations are formed between the successive ganglia themselves (Pl. XXXVIc, fig. 37). Here also Hatscheck asserts that the infolded cells give rise to the long commissures. When such an invagination has once been proved to exist, as it undoubtedly does, one does not have to go far for an explanation; in fact, that offered by Hatscheck is very plausible, and the only one at hand. But this is one of the cases where it is extremely easy to find a probable explanation, but very difficult indeed to prove that such is really the case. In other words, we lack the positive

proof that the invaginated ectoderm cells give rise either to the long or the short commissures.

Before the formation of the brain by the union of the thickened procephalic lobes, a deep fold is formed on the lateral surface of each lobe, which results in the formation of a thin blade of ectoderm, which imperfectly divides the brain into an anterior and a posterior portion. This fold is seen in surface view in Pl. XXXVIA, figs. 11 and 12, and a section of the same in Pl. XXXVIC, fig. 41. Although not indicated in fig. 41, since the stage is too late, there is an actual infolding of the ectoderm produced, which, according to Tichomerof ("Ueber die Entwick. des Seidenwürms," 'Zool. Anzeiger,' ii, Jahr. No. 20 (Preliminary Notice)), produces a part of the chitinous internal support for the brain, while, on the other hand, Hatscheck claims that it forms a part of the ganglionic cells of the brain. As an objection to Tichomerof's view, it can be urged that this fold is fundamentally different from the thickened rod-like ectodermic ingrowths which undoubtedly form a part of the internal skull (Pl. XXXVIB, fig. 16, *a*, and fig. 15, *d*). These unfoldings, however, are exactly like those between the post-oral ganglia, and hence lead to the conclusion that the brain is formed of two pairs of coalesced ganglia, from the posterior pair of which the antennæ are developed, the anterior pair producing no appendages. The principal objection to this view is that instead of having a single transverse fold as in the post-oral segments, we have in reality two separate, lateral infoldings, one on each side. The lateral position, however, is easily explained, since the procephalic lobes, originally strictly ventral in position, have been bent in a dorsal direction, giving them a lateral position as related to the germinal band. The fact, however, that there are two folds is a real difficulty, and can only be explained by assuming that the fold which originally extended along the whole ventral face of the brain has disappeared in the middle line, thus leaving two separate and lateral, although originally continuous, infoldings. We have, however, no facts to warrant such a supposition. If the brain in reality is made by the fusion of two pairs of ganglia, then

the antennæ which develop from the second pair of ganglia are homologous with the second pair of crustacean antennæ; structures corresponding to the first pair of crustacean antennæ are not developed in insects. Our knowledge of the development of the brain in Crustacea is as yet so imperfect as to forbid any comparison between its development and that of insects. I hope, however, to be able to make some investigation on this subject from a comparative point of view.

A cross section of the brain, perpendicular to the anterior surface of the head, discloses a pair of solid rod-like ingrowths of the ectoderm, which form a part of the endocranial support for the brain (Pl. XXXVI_B, fig. 16). There is no infolding at the surface to mark their points of origin. A second pair is shown in sections dorsal and parallel to that just described; they originate from the posterior wall, and project forward and inward (Pl. XXXVI_B, fig. 15).

Bridging a broad and deep invagination of ectoderm in the middle of the anterior face of the head, there is developed a low conical structure with a sharp apex. It is the same organ described by Zaddach as an apparatus used in rupturing the chorion at the time of hatching (Pl. XXXVI_B, fig. 15, *c*).

A very remarkable club-shaped organ lies just beneath the structure just described; at its lower end it is somewhat narrowed and is attached to the anterior surface of the brain; from this point it projects upward into the narrow space between the brain and its chitinous wall, and there terminates freely. It is bounded on each side by an infolded portion of the ectoderm, which forms a part of the endocranium. When seen in section (Pl. XXXVI_B, fig. 15, *b*) it appears like a tube whose wall is thrown into folds, the centre being occupied by a clearer and less granular substance. These two peculiar organs, which appear to stand in intimate relation with one another, I regard as the rudiments of the simple eye, the so called "egg-rupturing apparatus" being the modified lense (?) and the club-shaped organ beneath a special nerve centre, corresponding to the ring-like structures seen, according to Leydig, in the brain of ants. If, according

to Rathke and Zaddach, and Brandt among recent authors, this pointed organ serves to rupture the egg membrane—which I doubt very much, the only evidence of that purpose being in its apparent adaptability—its chief function probably does not lie in that alone, being merely a secondary adaptation of its primary function as a sense organ.

The compound eyes appear, just before the revolution of the embryo on the surface of the procephalic lobes, as round refractive areas, in which subsequently six dark red pigment spots appear (Pl. XXXVIA, figs. 13—16). A section through the eye at this period shows a thickened area of ectoderm, which ultimately becomes connected with the brain (Pl. XXXVIB, figs. 15 and 19).

Tracheæ are formed in all the post-oral segments, with the exception of the last two or three abdominal. The tracheal invaginations are less conspicuous in the abdominal segments than elsewhere; they are formed by an invagination of the ectoderm close to the nervous ganglia (Pl. XXXVIB, fig. 20, *t*). In the thorax and head the invaginations occur on the outer or dorsal sides of the appendages, the tracheæ thus formed lose their connection with the exterior, and, increasing in length in an antero-posterior direction, fuse with each other and form common tracheal trunks one on each side of the body.

The spinning glands are formed by a pair of ectodermic invaginations on the ventral side of the embryo, between the base of the second maxillæ and the nervous cord. They increase rapidly in length until they are nearly two thirds as long as the embryo, and when the second maxillæ fuse they also unite to form a common duct, which opens at the end of the upper lip.

The salivary glands are formed by invagination of the ectoderm on the inner sides of the mandibles, in the same manner as are the spinning glands. They are short tubes reaching a little back of the second maxillæ.

The mesoderm in the preceding stage had separated into segments, the lateral extremities of which were formed of two irregular layers of cells; no definite body cavity had appeared.

During the present stage the mesoderm increases in extent, and at the same time the cells composing it become less definitely and compactly arranged. The yolk, on account of its absorption by the growing embryo, becomes reduced in amount, and gradually recedes from the ventral plate. Into the cavity thus formed amoeboid mesoderm cells migrate, and give rise to the blood-corpuscles (Pl. XXXVI_B, fig. 20). As the yolk continues to withdraw from the ventral plate the mesoderm follows it by a rapid growth along its lateral margins, which, when they come in contact with the yolk, are reflected backwards and inwards towards the median line, thus forming, on either side of the body, a mesodermic layer, which bounds the yolk on the ventral side.

By continued growth the margins finally meet each other.

The mesoblastic wall, however, does not entirely separate the yolk from the body cavity, for it is segmented in such a way as to produce bands of mesoderm extending entirely across the ventral side of the yolk, alternating with other bands, which are interrupted in the middle line, thus allowing free passage from the yolk into the body cavity. A section through one of these interrupted bands is shown in Pl. XXXVI_B, fig. 20, where one or two yolk-cells are represented in the act of migrating from the yolk into the body cavity.

In the meantime the lips of the mesoblastic folds have progressed in an opposite direction, as shown by arrow No. 1 (Pl. XXXVI_B, fig. 20), giving rise to two layers of mesoderm, an inner and an outer. The inner layer or splanchnic mesoderm extends over and encloses the yolk, with its muscular layer before the yolk or endoderm cells have formed the epithelial lining of the stomach; the outer layer or somatic mesoderm becomes closely united to the ectoderm, with which it extends dorsally to form the outer wall of the sides and dorsum. Before the folds have met in the middle line of the dorsum the amnion and serosa rupture, so that the further consideration of the changes in these layers will be deferred until the next stage.

The endoderm cells increase very rapidly during this stage,

both in number and size. Their nuclei have become enormous, and it is only occasionally that one can distinguish protoplasm surrounding them. In size and general appearance they closely resemble granular yolk masses, and if their transition had not been traced step by step one would not believe that they were cells. Before the splanchnic mesoderm has completely separated the yolk from the body cavity on the ventral side some of the yolk-cells migrate into the body cavity, where they arrange themselves irregularly along the sides of the body wall. They are at once distinguished from the surrounding mesoderm cells by their great size and amœboid protoplasmic arms. At first the cells are arranged singly and indefinitely along the outer wall of the body; but later, by increase in numbers, they become arranged in distinct groups, which will be described more definitely in the next stage.

Exceedingly large vesicular cells, and probably of the same origin as those just described, are seen during this stage on the ventral side of the œsophagus, where it comes in contact with the yolk (Pl. XXXVIc, fig. 22, *en. c.*, and Pl. XXXVIc, fig. 36, *a*).

When the embryo is ready to hatch, these cells have disappeared, although their exact fate I was not able to determine with certainty.

In the latter part of this period the yolk-cells begin to arrange themselves upon the walls of the yolk-sac. During this process they become less distinct and gradually reduced in numbers, owing to the migration of some of them into the body cavity. In the anterior part of the yolk-sac it was extremely difficult to observe yolk-cells in the act of forming the epithelial lining of the stomach, since the cells were both so indistinct and few in number. On the other hand, it was comparatively easy to observe this in the posterior part of the yolk-sac, since the cells were both distinct and numerous; the epithelial lining of the stomach is probably formed first in the neighbourhood of the proctodæum, and then extends forwards to the stomodæum. It is certain, however, that the yolk-cells do not form a continuous sac until some time AFTER the formation of the mesodermic musculature of the yolk-sac. This

would account for the statement of Tichomerof, that the mesoderm gives rise to the epithelial lining of the mesenteron.

The Malpighian vessels (Pl. XXXVI_B, fig. 17) arise as THREE SEPARATE PAIRS of hollow outgrowths from the inner blind end of the proctodæum, one dorsal, one lateral, and one ventral; the latter pair appears first, and is somewhat larger than the others; from this pair also a fourth pair originates close to its points of origin. Further increase in the number of vessels is not produced by fresh evaginations of the proctodæum, but by budding from the first three pairs of Malpighian vessels.

Stage 6.—The time from the rupture of the embryonic membranes to that of hatching constitutes a sixth period. The most important changes which occur are—(1) the rupture of the embryonic membranes resulting in the formation of the dorsal organ; (2) the revolution of the embryo within the chorion; and (3) the formation of the heart.

At the beginning of this period the embryo assumes a peculiar shape, due to the fact that the abdomen has become bent back upon itself, a part of its ventral surface being now concave instead of convex. When the embryo has reached the condition represented in Pl. XXXVI_A, fig. 14, the amnion and serosa, which have become confluent or, at least, closely united, rupture along the line of confluence, the free edges of the membranes being reflected back upon the sides and dorsum; the rapidity with which they move towards the dorsal region indicating their elasticity and the high state of tension to which they have been subjected. They now concentrate on the dorsal portion of the yolk to form a rosette-shaped dorsal organ, which gradually disappears from the surface (Pl. XXXVI_A, fig. 15). A complete series of sections at successive stages (Pl. XXXVI_C, figs. 39, 40, 42) shows beyond a doubt that the dorsal organ sinks into the yolk and is absorbed. In the stages immediately following the retraction of the membranes the nuclei are very sharply defined, and are embedded in a clear mass of protoplasm in which is soon formed a tubular invagination nearly perpendicular to the dorsal surface of the yolk. A longitudinal section of this invagination is to be seen

in Pl. XXXVIc, fig. 39. The whole dorsal organ now sinks into the yolk, the nuclei become less distinct, and by the confluence of its sides the invagination disappears (fig. 40). Still later no trace of the nuclei can be detected; they have gradually faded, and finally disappeared altogether from the protoplasmic mass without shifting their position. The protoplasmic matrix in which the nuclei were embedded does not break up into fragments, but disappears en masse some time after the nuclei.

After the rupture of the membranes, and during the changes which take place in the dorsal organ, the abdomen of the embryo becomes folded more and more towards the ventral side, until the curvature of the embryo becomes completely reversed (compare Pl. XXXVIA, figs. 12 and 16).

After the dorsal organ has passed into the yolk, the lips of the uprising mesoderm, which on either side unite the splanchnic and somatic layers, gradually envelop the yolk, and finally meet each other along the middle line of the dorsum. By the fusion of these two mesodermic lips a solid cord of cells is formed occupying the median longitudinal line of the back indicating the first foundation of the heart. A cross section of the heart at this period is represented in Pl. XXXVIc, fig. 32, from which it will be observed that the cells have fused into a common mass of protoplasm in which the nuclei are irregularly arranged.

Transverse sections through the embryo just before hatching show that this cord of cells has become hollow; the cavity thus formed is the lumen of the dorsal blood-vessel.¹ I am unable to say whether any of the nuclei of this cord are isolated and fall into the lumen during the histological differentiation of its walls which are formed by the production of cells from the syncytial mass. These cells give rise to successive muscular rings, each of which is composed of two transversely striped muscle cells, each cell forming one of the lateral halves

¹ It was probably this similarity which led Weismann to think that the heart of the fly was formed by the hollowing of a solid muscular cord.

of the ring and containing near its centre a single nucleus (Pl. XXXVIc, fig. 30).

Just before the embryo hatches all the yolk-cells have migrated to the periphery of the yolk, where they arrange themselves to form the epithelial lining of the mesenteron. About the same time the thin membranes at the blind ends of the sternodæum and proctodæum rupture, and their cavities become continuous with that of the mesenteron.

The remaining organs of the body which were already formed during the preceding stages do not undergo any important changes during this period.

THE LARVA.

The larva, which resembles that of *Mystacides* figured by Zaddach, immediately after its escape from the egg forms a case of fine sand and small fragments of vegetable matter.

In larvæ several weeks old no gills were present unless the vesicular appendages between the anal stylets can be considered as such. When, however, the larva is about three sixteenths of an inch long a pair of filamentous tracheal gills bud from the lateral walls of each segment of the abdomen.

Sections of very young larvæ show the complete formation of all the larval organs. The body cavity is broken up into irregular spaces bounded by thin membranes, on the walls of which there may be seen a number of large cells whose protoplasmic filaments extend across the cavity, and either unite with the opposite wall or with similar filaments from other cells (Pl. XXXVIc, fig. 29). These cells are the same that we have already observed in the preceding stages attached to the body wall, and which had migrated into the body cavity from the yolk-sac.

The salivary glands have already assumed the peculiar and characteristic appearance that they have in the adult. A cross section through one of them (Pl. XXXVIc, figs. 25 and 26) shows in the centre an exceedingly small duct surrounded by a zone of radiating filaments; outside the latter, and constituting

the remainder of the section, there is a wide zone of a finely granular substance in which one may observe scattered nuclei.

SUMMARY.

The most important results at which I have arrived may be summarized as follows:

In the earliest stages observed there were already a number of germ-cells in the yolk, together with an irregular network of protoplasm. ALL THE NUCLEI and protoplasmic network migrate to the surface and form a syncytium or "blastema," which, by the segregation of definite masses of protoplasm around each nuclei, is converted into the "blastoderm," which then becomes thickened at one pole to form the "ventral plate."

From any point of the ventral plate, or serosa, cells arise by budding and migrating into the yolk and form the so-called "yolk-cells," the greater part of which ultimately form the epithelial lining of the mesenteron, and hence are to be regarded as true entoderm cells which have arisen by DELAMINATION from the ectoderm. After the segmentation of the mesoderm certain cells were observed in stages of separation from the mesodermic segments, which probably migrate into the yolk, and then cannot be distinguished from the ordinary yolk-cells.

During the formation of the embryonic membranes there is formed a median longitudinal invagination, resulting in the production of a continuous plate of mesoderm. The groove formed by this invagination quickly disappears, and then follows a stage in which the ventral plate has no median depression. Very soon, however, a SECOND DEPRESSION originates along the same median line, which initiates the formation of the nervous system. The latter is formed by the differentiation of a pair of lateral cords arising from the division of the ectoderm cells lying on either side of the neural furrow, and by the addition of a median infolded portion of the ectoderm, which may possibly form the cross commissures. There are also transverse infoldings of the ectoderm between the contiguous segments.

The mesoderm separates early along the median line, forming a pair of lateral bands, which soon break up into segments, in each of which arises an imperfectly closed space, the body cavity. The splanchnic mesoderm grows under the yolk in alternating, continuous, and interrupted bands. Through the openings in the interrupted bands some of the yolk-cells pass into the body cavity. Before the embryonic membranes rupture the yolk-cells have already begun to form the lining of the posterior part of the mesenteron.

Tracheæ are formed in all the post-oral segments, except the last two or three segments of the abdomen.

The spinning and salivary glands are formed by special ectodermic invagination on the inner side of the second maxillæ and the mandibles respectively.

The Malpighian vessels arise as six separate evaginations of the blind end of the proctodorum.

The embryonic membranes, after rupturing, retreat towards the back, where they form the dorsal organ, which at one stage is simply a vertical tube-like invagination that quickly disappears, and the whole mass of cells which constitute it gradually sink into the yolk and is absorbed.

A solid cord of cells is formed in the median dorsal region by the fusion of the edges of the mesodermic folds. The cord becomes hollow and forms the heart.

CONCLUDING REMARKS.

No group in the animal kingdom shows such a wonderful morphological and physiological diversity as the Tracheata, and this tendency to vary is as strikingly manifested in the development of the individual as in the adults themselves. Even closely related groups present the widest variations in their fundamental processes of development. Hence the few facts obtained from the development of one species do not warrant wide generalizations.

The variation in the amount of yolk always affords a convenient and often a very plausible explanation for these modified processes of development, but it will not account for everything,

and in many cases we must look beyond this for an adequate explanation of the facts.

The yolk doubtless plays a very important part in modifying the segmentation of the egg; the amount is so great that if it were intimately combined with the protoplasm it would prove a very serious obstacle to segmentation, and hence to accelerate development it is from the first entirely separated from it.

We may conceive that the germinative vesicle is surrounded by an irregular mass of protoplasm, scattered widely through the yolk. When the nucleus divides, only a small portion of the protoplasm collects around the resulting nuclei, the remaining portion being irregularly distributed throughout the yolk. After a number of germinative cells have been formed exactly the same conditions would exist that we have first observed (Pl. XXXVI B, fig. 1).

Both the germ-cells and outlying protoplasm are subjected to some centrifugal influence which propels them towards the surface. This might give rise to different results, varying with the amount and condition of the yolk. It would be a very slight and unimportant modification of the more ordinary course of events to have the network of protoplasm reach the periphery first, and thus form a superficial protoplasmic layer before the less mobile nuclei could migrate through the yolk and reach the surface. The impediment offered to the nuclei by the yolk would easily account for the presence in some insects (*Musca*, *Chironomus*, &c.) of what Weismann named the *Keimhautblastem*, whereas the absence or diminution of this impediment would in other cases (e.g. *Aphis*, &c.) allow the nuclei to arrive at the surface simultaneously with the accumulation of protoplasm. The Phryganids offer a condition which is intermediate between these extremes.

One of the most remarkable results due to the presence of so much yolk is the production of three distinct stages in the segmentation of the egg; first, the germinative vesicle with its protoplasm divides and produces a number of cells which unite at the surface to form a syncytium; second, the syncytium quickly divides into a number of cells with cell walls; and third,

long after the germinal band is formed, and the appendages have appeared, the yolk itself undergoes a cleavage of greater or less extent. The attempt has been made to show that the cleavage of the yolk is due to some centripetal force of the yolk-cells; if this was the only cause we would have one of two results dependent upon the relative strength of this centripetal force and the inertia of the yolk particles. If, on the one hand, the force is strong enough to overcome the inertia of the yolk particles, yolk-balls will be formed around each nucleus; this indeed is often the case, as, for instance, in *Lina* and *Hydrophilus*, and certain *Phryganids*. On the other hand, if this force is not strong enough to overcome the inertia no cleavage will take place. In other words, provided the force of each nucleus is the same, and they are regularly distributed, either a complete cleavage into a number of segments equal to the number of nuclei will take place, or no cleavage at all will result. For instance, the condition shown in *Neophalax* would never be realised by such a disposition of forces; for in this case the yolk is simply divided into two or three segments in which there are a great many nuclei. However, in *Mystacides* (Zadach) there are no more yolk-cells present than in *Neophalax*, yet there is a complete segmentation of the yolk. There is no doubt that a division of the yolk by increasing the exposed surface would greatly enhance its transformation and absorption by the embryo, and for that reason, other things being equal, we might expect to find a definite relation existing between the extent of segmentation and the duration of the development. This, however, does not appear to be the case, for in *Æcanthus* (one of the *Locustina*) the yolk is completely segmented, and the eggs which are laid early in the fall do not hatch until the next summer; while in *Blatta germanica* the yolk is very slightly segmented, and the development lasts but a few weeks. At present, therefore, a great many more facts are necessary before it will be possible to discuss the question with any probability of arriving at a definite conclusion.

It seems very probable that in all insects at least a large portion of the mesoderm arises by an infolding along the

median line of the ventral plate. This has been observed in all cases where the development has been carefully studied. Kowalevsky was the first to call attention to it, and his observations have been confirmed by Graber, 1877, and the Hertwigs, 1881. In the Phryganids, all the mesoderm arises from this invagination. Tichomeroff, 1879, claims that the mesoderm is not formed by an invagination, but by budding from the cells of the blastoderm and primitive ventral plate. Since the gastrula stage is of such short duration, it was probably not included in the eggs which he sectioned, and the cells observed to bud from the ventral plate and blastoderm are in reality endoderm, instead of mesoderm cells.

Graber had already claimed, in 1871, that some of the endoderm cells were formed from the blastoderm. Balfour, however, discredits his statements and believes that the phenomena observed by Graber were in reality due to the passage of yolk-cells into the blastoderm. It is very probable, however, that Graber is right in his conclusions, and that he observed the same process that we have described for Phryganids. In this case, however, we have more conclusive evidence, for ALL THE GERM CELLS having migrated to the surface, it is much easier to observe a passage of cells from the blastoderm into the yolk. In the later stages, where yolk-cells apparently bud from the mesodermic segments, it is not possible to state with the same confidence the direction in which the cells are migrating. The facts, however, seem to indicate, as we have already stated, that some of the yolk-cells are formed at this late period by budding from the mesodermic segments. This does not appear so improbable when we reflect that the segmentation of the yolk, a process which ordinarily precedes the formation of the endoderm, occurs also at this period.

The dorsal organ, or something equivalent to it, is found in all insects whose development has been carefully investigated. Uljanin and Bobretzky have described the dorsal organ of Amphiphods as a saddle-shaped area of cells attached to the vitelline membrane, and extending over the dorsal region of the body; the whole layer becomes disconnected from the

embryo except by a central umbilicus. It finally, however, becomes completely separated, and in *Orchestia* sinks into the yolk.

As Bobretzky has hinted, it is very probable that this saddle-shaped area of cells is homologous with the embryonic membranes of insects. If we imagine, for instance, that the ventral plate of *Oniscus* had extended nearly around the egg and then the embryonic membranes formed just as in insects (without, however, extending completely over the ventral surface of embryo), and if the membranes thus formed should fuse with the vitelline membrane, we should then have an organ which would be like the dorsal organ of *Oniscus*, and at the same time homologous with the embryonic membranes of insects. If, to carry the comparison still farther, the dorsal plate of cells should separate from the vitelline membrane, and, contracting, sink into the yolk, we should have still further grounds for comparison. In fact these conditions are realised in embryo Amphipods and Isopods, and show that the saddle-shaped plate of cells in *Oniscus*, &c., undergoes a series of changes, parallel with those of the embryonic membranes and dorsal organ of insects. A careful distinction, however, should be made between the dorsal organ of *Insecta*, and that of the Phyllopods and Nauplei of Decapods, &c., all of which have been considered by Dohrn as homologous structures. As a matter of fact, however, the only points they have in common is that all are located in the neighbourhood of the dorsum and very little is known about them.

It will be observed from the description already given of the germinal band with its gastrula and neural invaginations that a wonderful analogy at least, if not homology, exists between these structures and the corresponding ones in Vertebrates, and especially birds. For instance, in both bird and insect the germinal band has the same slipper-shaped outline. The primitive streak of birds and the gastrula in insects, both of which are homologous structures, begin at the posterior end of the germinal band and extend forwards. Likewise the medullary furrow in both groups arises at the anterior

end of the germinal band, and extends towards the posterior extremity, the only difference being that in insects the medullary or neural furrow is formed in nearly the same place where the gastrula occurred, while in birds the medullary furrow is located somewhat in front of the primitive streak.¹

Still another point of comparison between the nervous system of Vertebrates and insects is shown by the fact that in both groups a differentiation into two kinds of nerve-cells occurs—an inner layer with granular nuclei, and an outer layer, composed of faintly-marked nuclei. The former layer corresponds to the grey nerve-matter of Vertebrates, and the latter to the white substance. This differentiation in structure indicates the same physiological differentiation of the nervous system in both groups.

Blatta Germanica.

Since the completion of the paper on *Neophalax*, a few observations have been made upon the development of *Blatta Germanica*, some of which may be mentioned here preliminarily, reserving a more detailed and complete account until the observations on this form have been completed.

In the embryos of *Blatta* as well as in those of most if not all other insects, there appears in each of the segments at a certain time a great number of clear, highly refractive particles that at first might be taken for oil globules, and which have always been regarded as such. On more careful exami-

¹ The gastrula in insects disappears from the anterior end of the germinal band, backwards; while the medullary or neural furrow, which commences somewhat in front of the anterior extremity of the gastrula, is forming in the same direction; before, however, the gastrula has entirely disappeared from the posterior extremity the medullary furrow has extended to the posterior end, where it opens into the gastrula, both becoming continuous and forming a NEURENTRIC CANAL, just as in birds and other Vertebrates with meroblastic ova.

nation, however, it will readily be seen that this supposition is incorrect. A number of tests have been made in order to ascertain the nature of these bodies, and the results show that they are some salts of uric acid. That they are not of a fatty nature is indicated by the fact that treatment of the embryos with hot benzole, chloroform, or clove oil has not the slightest effect upon the bodies in question. Further examination with a highly magnifying power shows that they consist of small spheres of an extremely refractive substance, from the centre of which dark lines radiate in an irregular manner, producing the same appearance seen in the crystals of urea from the Malpighian vessels. It was this similarity which first suggested the true nature of these bodies, and further tests confirmed this view, for after heating an embryo with nitric acid upon a glass slide, and then adding a little ammonia, the characteristic red colour of Murexid was formed. A still further test was used by dissolving the granules in dilute caustic potash, and then precipitating the urea by adding acetic acid, although this method did not give such definite results as the first.

A rather striking variation was found in the first and second maxillæ of *Blatta*, which were formed respectively of two and three branches, the second maxillæ thus attaining the typical trichotomous structure of the Crustacean appendages.

At first a number of abdominal appendages are developed, which, however, quickly disappear again with the exception of the first pair, which further develops into pear-shaped structures attached to the abdomen by a stem that increases in length and finally changes into a very fine duct leading into a small cavity in the expanded distal extremity, which owes its size to the development of extremely high ectoderm cells. No mesoderm enters into the construction of this peculiar organ, which in the later stages of development disappears entirely. This organ is undoubtedly a special development of the first abdominal appendages, yet its function is extremely problematical. Rathke observed what is very likely a similar organ in the embryos of *Grylotalpa* and which he regarded as a rudimentary gill, but the remarkably thick ectodermic walls of this structure makes

this supposition improbable. It is more likely that it had some sensory (?) function on account of the peculiar structure and development of the ectoderm cells, while at the same time the longitudinal duct leading into a cavity into which the large cells could pour a secretion points to the supposition that it is possibly of a glandular nature.

In *Blatta* the heart is formed by the fusion of mesodermic folds similar to those in Phryganids; instead, however, of forming a solid cord the heart is from the first hollow. The mesodermic folds pulsate regularly long before they have united to form the heart.

NOTE.

Since the completion of this paper on *Blatta* and Phryganids the work of Dr. A. Korotneff on the development of the heart in *Gryllotalpa* has appeared ('Zool. Anzeiger,' December 24th, 1883), which contains several points on the origin of the heart and blood-corpuscles resembling what I have found in *Blatta*. The blood-corpuscles both in *Blatta* and Phryganids are formed from free mesoderm cells, which, however, do not arise in the median line of the embryo, as this author has indicated, but from the apex of the angle made by the united splanchnic and somatic mesoderm.

The condition shown in *Gryllotalpa*, in which the semicircular projections on the uprising mesodermic folds are formed before the folds have hardly begun to grow towards the back, is not attained in *Blatta* until the folds have very nearly united with each other in the median dorsal line. Moreover, a thin layer of mesoderm always closes in the yolk dorsally before the mesoderm folds unite, thus preventing a communication between the blood system and the yolk-sac, as Tichomerof has described in *Lepidoptera*.

I cannot believe that the condition represented by Korotneff is possible. In the first place it is improbable, on theoretical grounds, that a blood sinus should be formed simply between the vitelline membrane and the yolk, as he described. In fact,

I do not believe such an extended space as he described exists at all, unless produced by shrinkage due to reagents. The yolk is separated from the vitelline membrane by the upgrowing mesoderm folds, and the only space formed is just before the folds unite to form the heart, and this space is simply the cavity of the heart.

The primitive blood sinus is not the space between the lips of the mesoderm folds, however large or small this may be, for this space is not in communication with the body cavity at all, and even when the mesodermic folds first begin to beat the space contains no blood-corpuscles. The primitive blood sinus, then, is the space between the somatic and splanchnic mesoderm, divided into a number of smaller and irregular sinuses by meshes of connective tissue, some cells of which, in the earlier stages, become free and form the blood-corpuscles. By the pulsation of the mesodermic folds, long before a special heart is formed, a circulation through the irregular sinuses of the body cavity is brought about, like the circulation in many of the lower Worms.

By the union of the mesodermic folds a definite heart is formed, from whose walls, in the earlier stages, a number of blood-corpuscles are produced in addition to those formed in the true body cavity. I hope to produce absolute proof of this statement, accompanied by drawings of cells in different stages of separation from the dorsal inner wall of the heart, when all my observations on *Blatta* are completed and published. These facts add the evidence of embryology to that of comparative anatomy, to show that the heart of Worms and Arthropods is a comparatively new structure, which was preceded by a circulation produced by contraction of the muscular walls of the body cavity.

EXPLANATION OF PLATES XXXVIA, XXXVIB,
XXXVIC.

Illustrating Mr. W. Patten's paper on "The Development of Phryganids, with a Preliminary Note on the Development of *Blatta Germanica*."

List of Reference Letters.

am. Amnion. *an.* Anus. *at.* Antenna. *b. cv.* Body-cavity. *bd.* Blastoderm. *bl.* Blastema. *br.* Brain. *cm.* Cross commissures. *d. o.* Dorsal organ. *ec.* Ectoderm. *en.* Endoderm. *f. hd.* Fore head. *ht.* Heart. *l. cm.* Long commissures. *md.* Mandible. *mp. v.* Malpighian vessels. *ms.* Mesoderm. *mx.¹* and *mx.²* Maxillæ 1 and 2. *n. f.* Neural furrow. *œ.* Œsophagus. *p.^{1 2 3}* Thoracic appendages. *pc. l.* Procephalic lobes. *pr.* Proctoderm. *sl. g.* Salivary glands. *sp. g.* Spinning glands. *sr.* Serosa. *tr.* Trochæ. *v. p.* Ventral plate. *y. c.* Yolk-cells. *gn.* Ganglion.

PLATE XXXVIA.

FIG. 1.—Lateral view of the egg in the blastoderm stage.

FIG. 2.—Same view of the egg four hours later, showing the thickening of the blastoderm to form the ventral plate.

FIG. 3.—Lateral view of the ventral plate during the formation of the embryonic membranes. It is shown as a semi-transparent mass of cells; the headfold at *am.*, and tailfold at *a*.

FIG. 4.—Same embryo one hour later, the head and tailfold have approached each other preparatory to uniting.

FIG. 5.—Dorsal view of the anterior end of the embryo. The amnion and germinal band are seen in optical section. *gst.*, gastrula invagination.

FIG. 6.—Same view of the embryo, after the gastrula invagination has disappeared. The embryo has increased in length, so that the ventral plate extends the whole length of the egg. At *a* is a segmentation furrow dividing the yolk into halves.

FIG. 7.—Lateral view of the embryo, seen from the left side. None of the appendages have as yet appeared.

FIG. 8.—The same embryo twenty-four hours later, showing the first appearance of the appendages.

FIG. 9.—Same embryo, two days later than that represented in Fig. 7. The ventral plate is seen to be continuous with the amnion at both extremities of the body, *am.¹* and *am.²*

FIG. 10.—Lateral view of the embryo in about the middle of the fifth stage.

FIG. 11.—Same embryo two days later. At *p.*⁴ ⁵ ⁶ may be seen the rudimentary abdominal appendages; *a. st.*, anal stylets in optical section; *ft.*, bodies, which in *Blatta* we have shown to be salts of urea, and which are undoubtedly of the same nature here.

FIG. 12.—Lateral view of an embryo about twelve days old. The tip of the abdomen is bent back as far as the eighth abdominal segment.

FIG. 13.—Lateral view of the embryo in the latter part of the fifth stage. The tip of the abdomen has reached the sixth abdominal segment.

FIG. 14.—Embryo, just before the rupture of the embryonic membranes.

FIG. 15.—Lateral view of the right side. Embryo in the last stages of revolution, the membranes have ruptured and collected on the back to form the dorsal organ, *d. o.*

FIG. 16.—Lateral view of the embryo from the right side, after the completion of revolution.

PLATE XXXVIB.

FIGS. 1, 2, and 3.—Three successive stages in the formation of the "blastema."

FIG. 4.—Longitudinal section through the egg in blastoderm stage.

FIG. 5.—A similar section during the formation of the ventral plate. *a.* Yolk-cells in the act of separating from the serosa.

FIG. 6.—Section through a mesodermic segment, showing two cells in the act of separating from the mesoderm and passing into the yolk.

FIG. 7.—Longitudinal section of the ventral plate, showing another cell in the act of separating from the mesoderm.

FIG. 8.—Longitudinal section through the ventral plate, showing the formation of the embryonic membranes. At *y. c.* and *x.* are two cells in different stages of separation from the ventral plate and serosa.

FIG. 9.—Cross section of ventral plate in head region, showing the gastrula invagination at *gst.*

FIG. 10.—Section similar to that in Fig. 9, only in a later stage. The embryonic membranes have united over the median line of the ventral plate. The gastrula invagination has disappeared, leaving a group of median cells behind.

FIG. 11.—Cross section in thoracic region before the appendages have appeared. The mesoderm has spread out into almost a single layer of cells, *ms.* The gastrula has entirely disappeared.

FIG. 12.—Cross section through the ventral plate during the early stages of the nervous system. A few nuclei have appeared at the deep ends of the ectoderm cells, on either side of the neural furrow. The mesoderm has separated from the median line and is thickened at the outer margins.

FIG. 13.—Cross section of the ventral plate in a later stage. Beneath the median furrow is a group of cells uniting the lateral cords.

FIG. 14.—Cross section of the abdomen during the stage represented in Pl. XXXVIA, fig. 12, showing a section of the proctodarium and the Malpighian vessels.

FIGS. 15, 16—19 are successive sections of the head in a plane perpendicular to the front wall of the cranium. Fig. 16*d* shows a pair of ectodermic rods forming a part of the endocranium. Fig. 15 is a section dorsal to the one just described; *c*, the so-called egg-rupturing organ, whose function, however, is still very doubtful, and which may represent the simple eye of Insects; *b*, a structure, probably of a ganglionic nature, connected with the simple eye; ectodermic ingrowths are developed on either side of these organs. Fig. 19, a section dorsal to that in Fig. 15, showing at *a* the ectodermic thickenings to form the eye; *en. c.*, a group of vesicular endoderm cells on the ventral side of the œsophagus.

FIG. 17.—Cross section of the blind end of the proctoderm, showing the three separate pairs of invaginations to form the Malpighian vessels.

FIG. 18.—Cross section of the folded abdomen in the region marked *an. st.*, Pl. XXXVIA, fig. 12. *a* is the median infolded portion of the ectoderm uniting the lateral cords; *a. st.*, anal stylets.

FIG. 20.—Cross section of first abdominal segment. *en. c.*, yolk-cells migrating into the body cavity; *so. m.*, somatic mesoderm; *sp. m.*, splanchnic mesoderm. The arrows indicate the directions in which the mesodermic folds are growing.

PLATE XXXVIc.

FIG. 21.—Cross section through the first maxillæ and procephalic lobes. In the deep surface of the lateral cords dark granular cells appear, which finally replace the more lightly stained ones. The stage corresponds to that shown in Pl. XXXVIA, fig. 10.

FIG. 22.—Longitudinal section of embryo corresponding to Pl. XXXVIA, fig. 12.

FIG. 23.—Cross section through the mandibular segment of an embryo corresponding to Pl. XXXVIA, fig. 12.

FIG. 24.—Cross section through abdomen of larva. *a*, segmentally arranged thickenings of the hypodermis; *y*, one of the wandering endoderm cells.

FIG. 25.—Cross section of salivary gland of larva.

FIG. 26.—Longitudinal section of a part of the same gland.

FIG. 27.—Muscle cells of the larva.

FIG. 29.—Section of one of the large cavities in the body of the larva. *d*, amœboid endoderm cells attached to its wall.

FIG. 30.—Cross section of the larva, showing the fully formed heart, *ht.*, and the wall of the mesenteron.

FIG. 31.—Part of a section through a larva. *a*, segmental thickening of the hypodermis opposite the tracheal trunk, *tr.*

FIG. 32.—Section through dorsal region of an advanced embryo, showing thickened cord of cells from which the heart is to be formed. *e. n.* yolk-cells, attaching themselves to the wall of the yolk-sac to form the mesenteron.

FIGS. 33, 34, and 35.—Very highly magnified cells of the ventral plate, showing various kinds of nuclei.

FIG. 36.—Cross section of the embryo through the first pair of maxillæ, showing the tracheal invaginations; at *a* are very large endoderm cells.

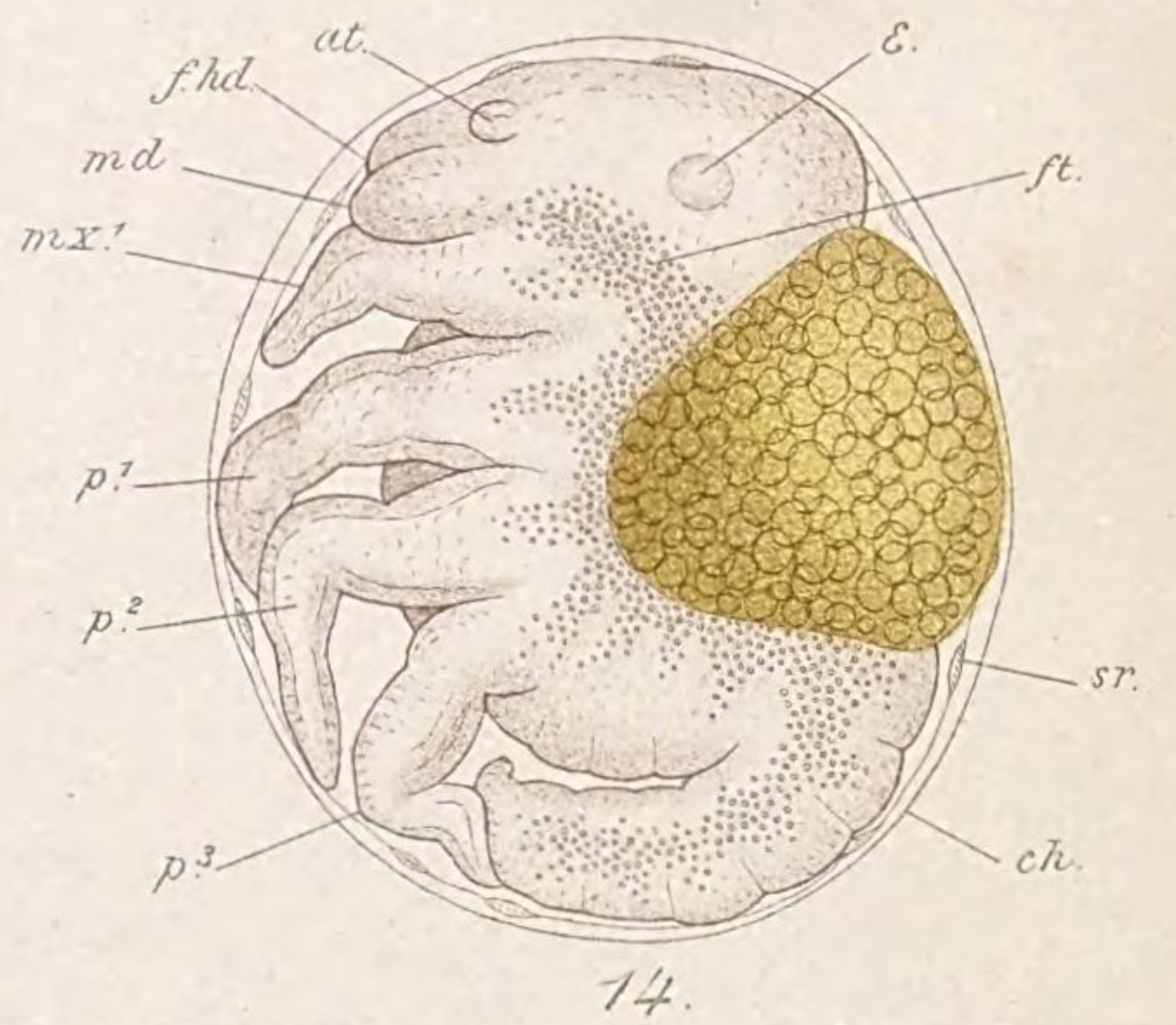
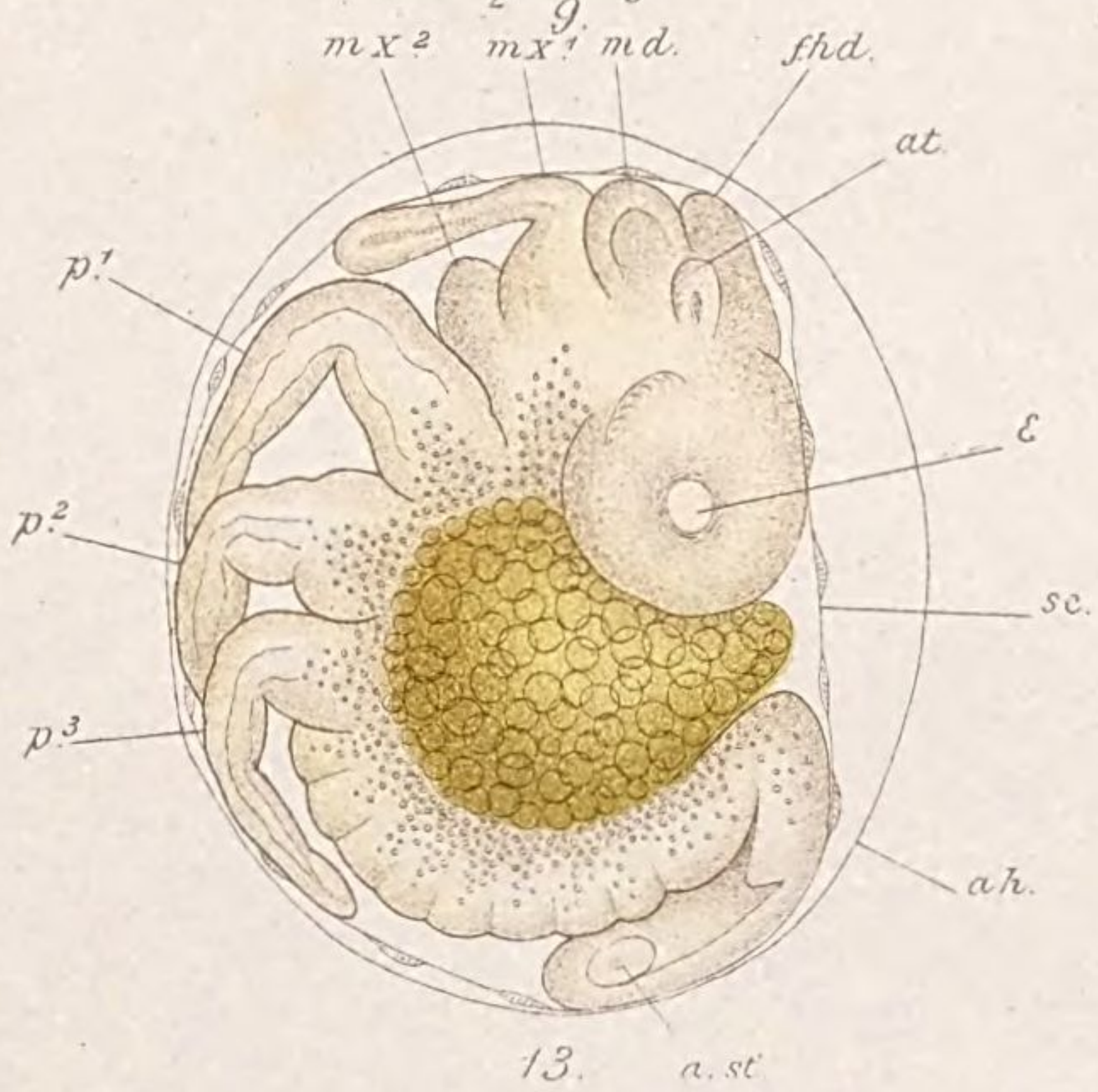
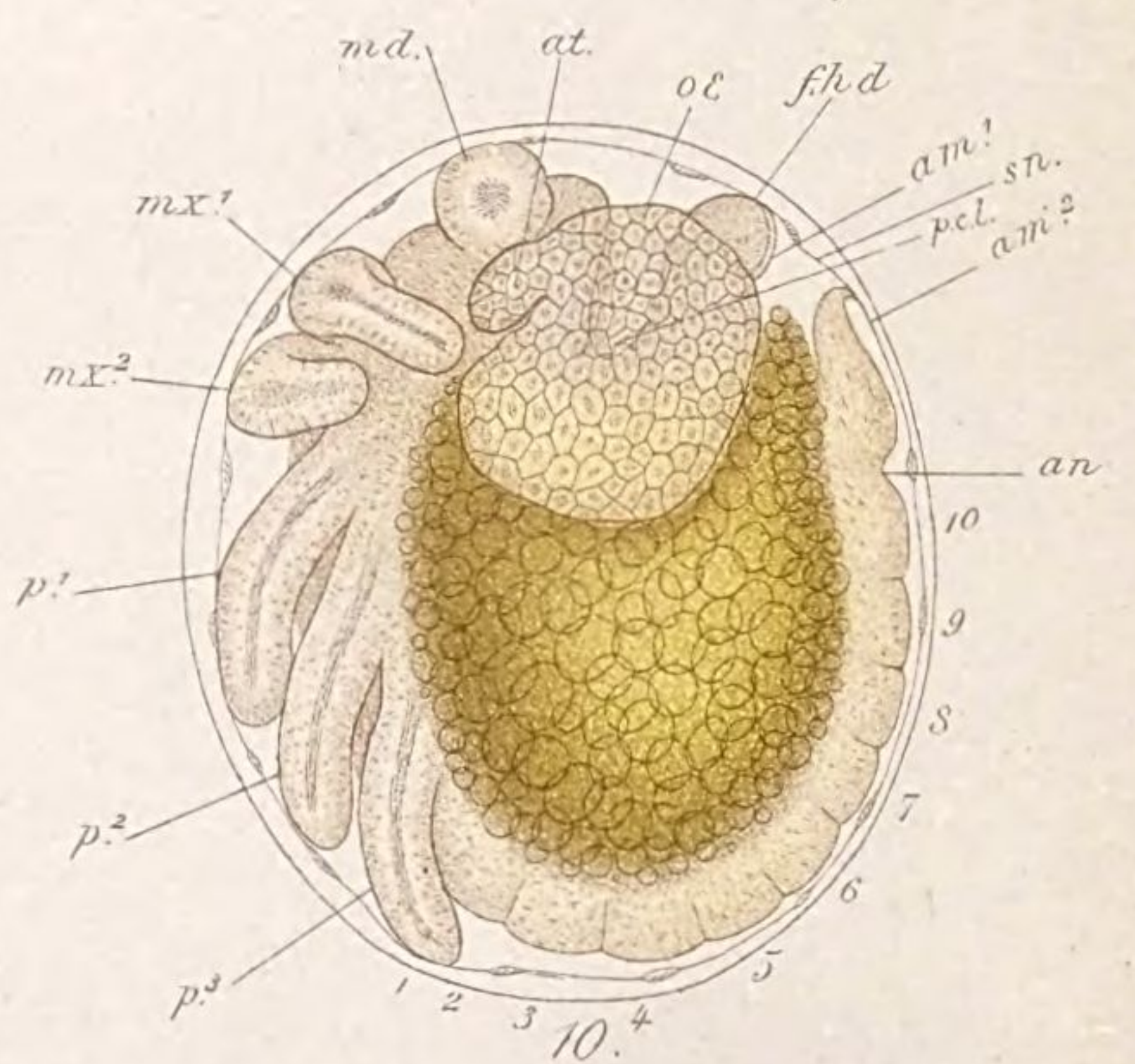
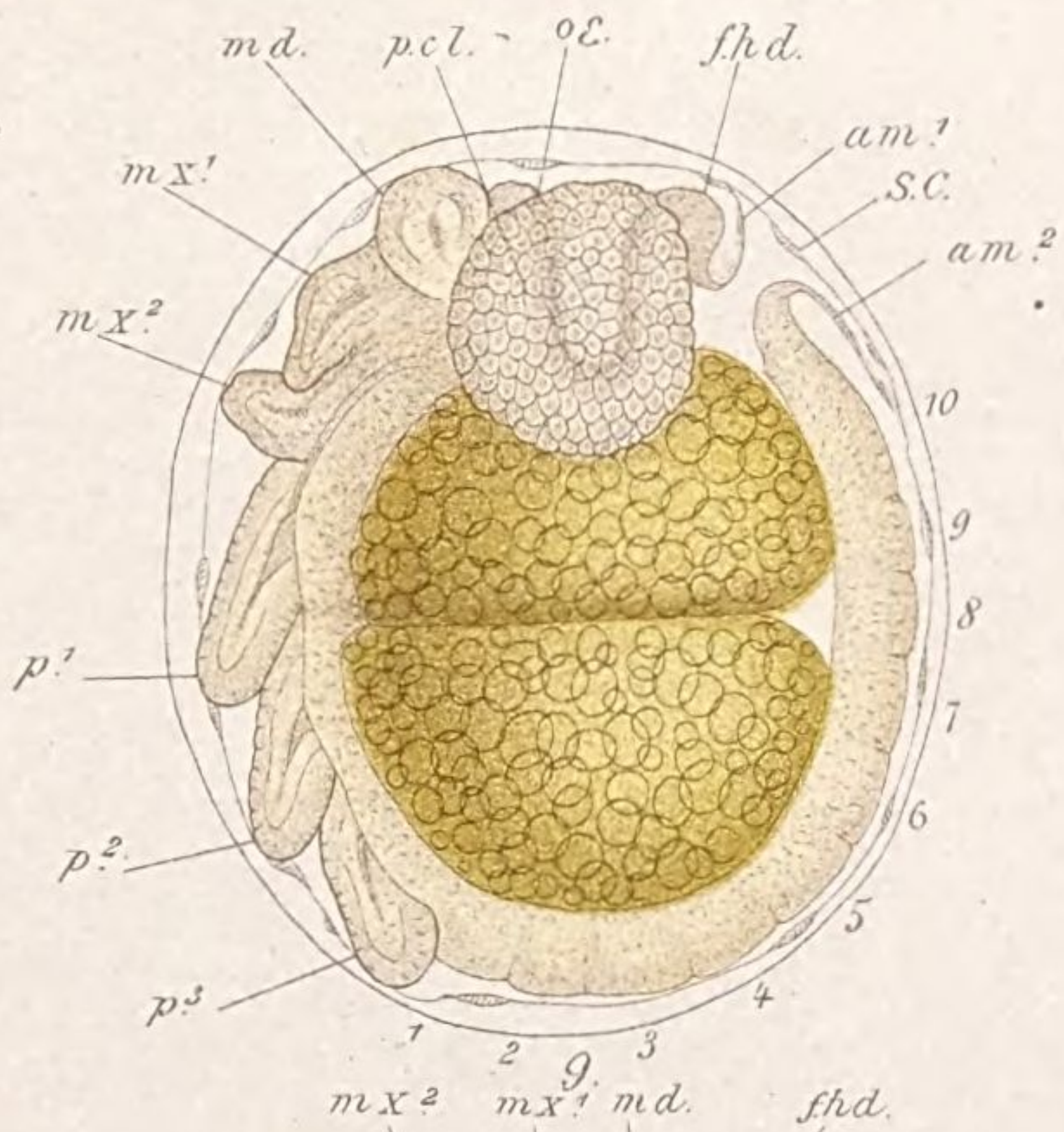
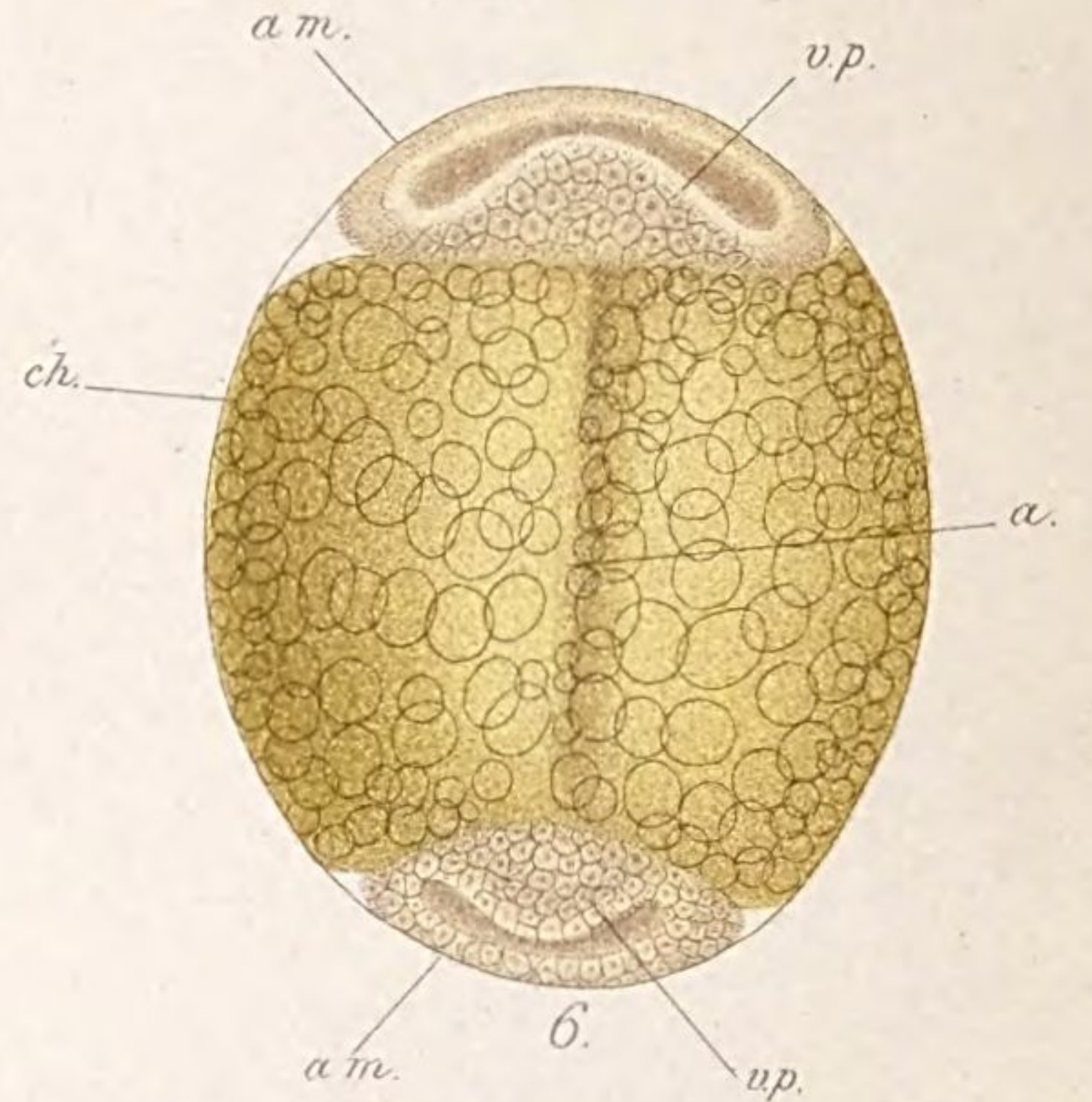
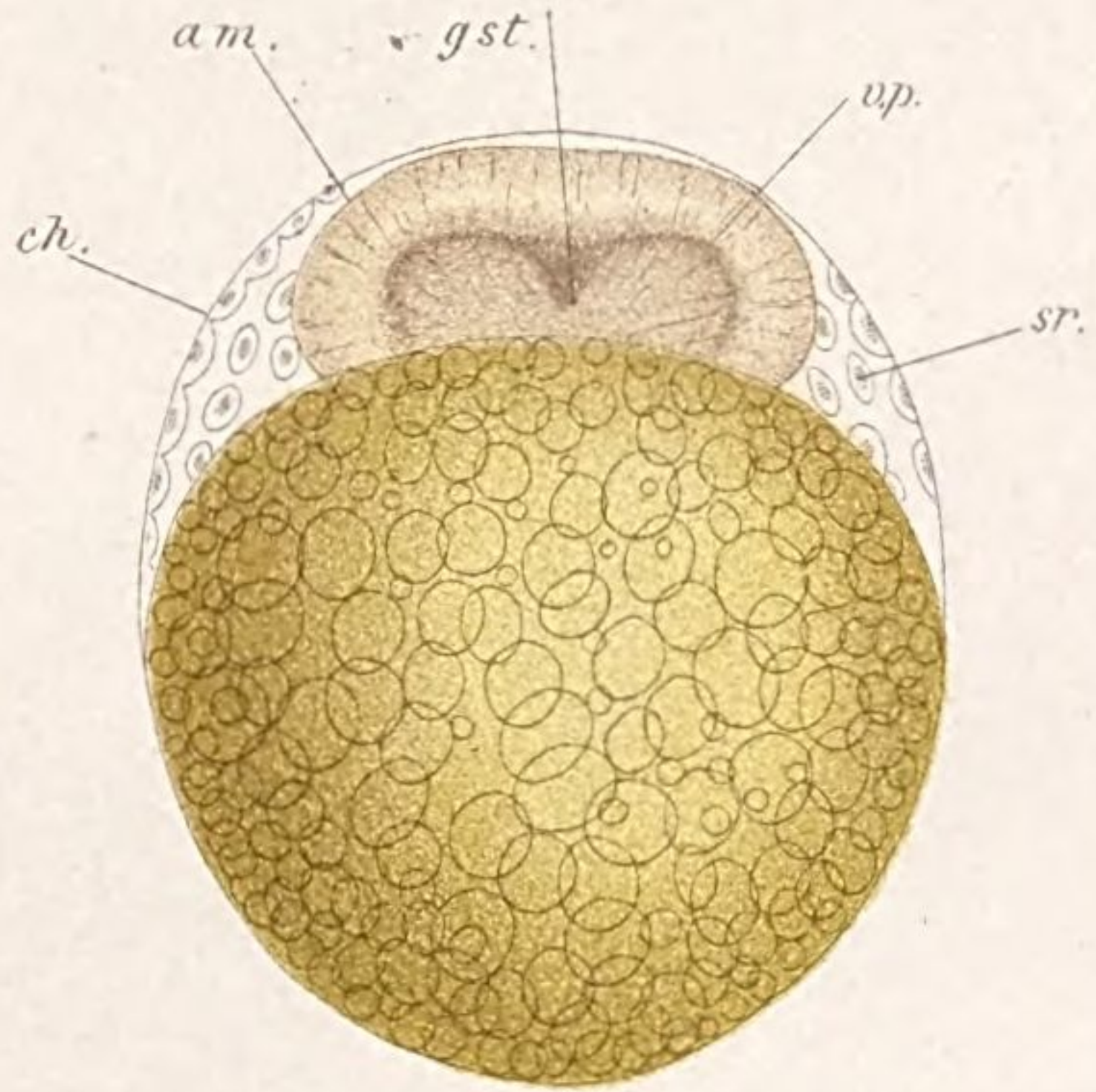
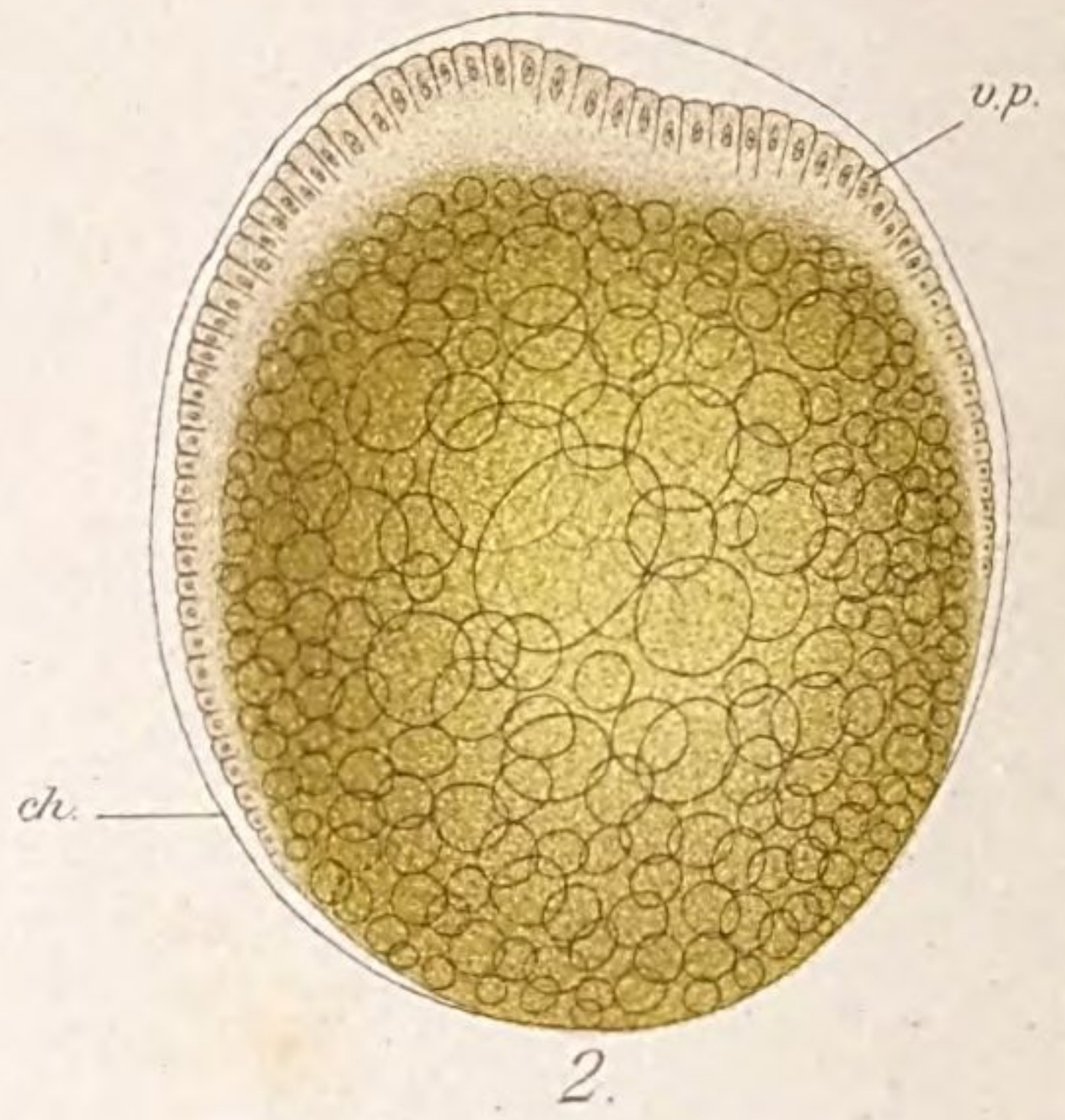
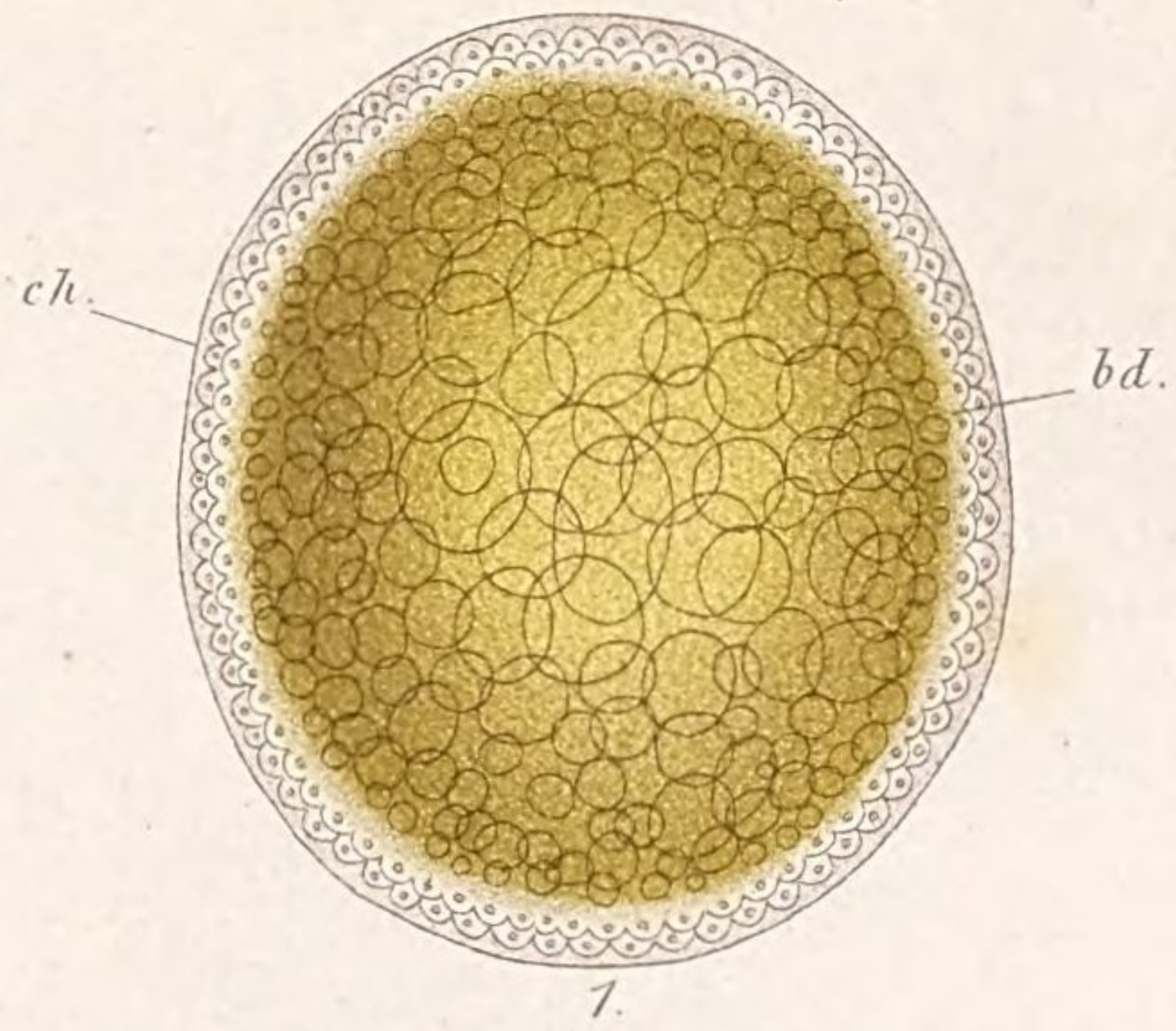
FIG. 37.—Sagittal section of the three thoracic ganglia, showing the double cross commissures.

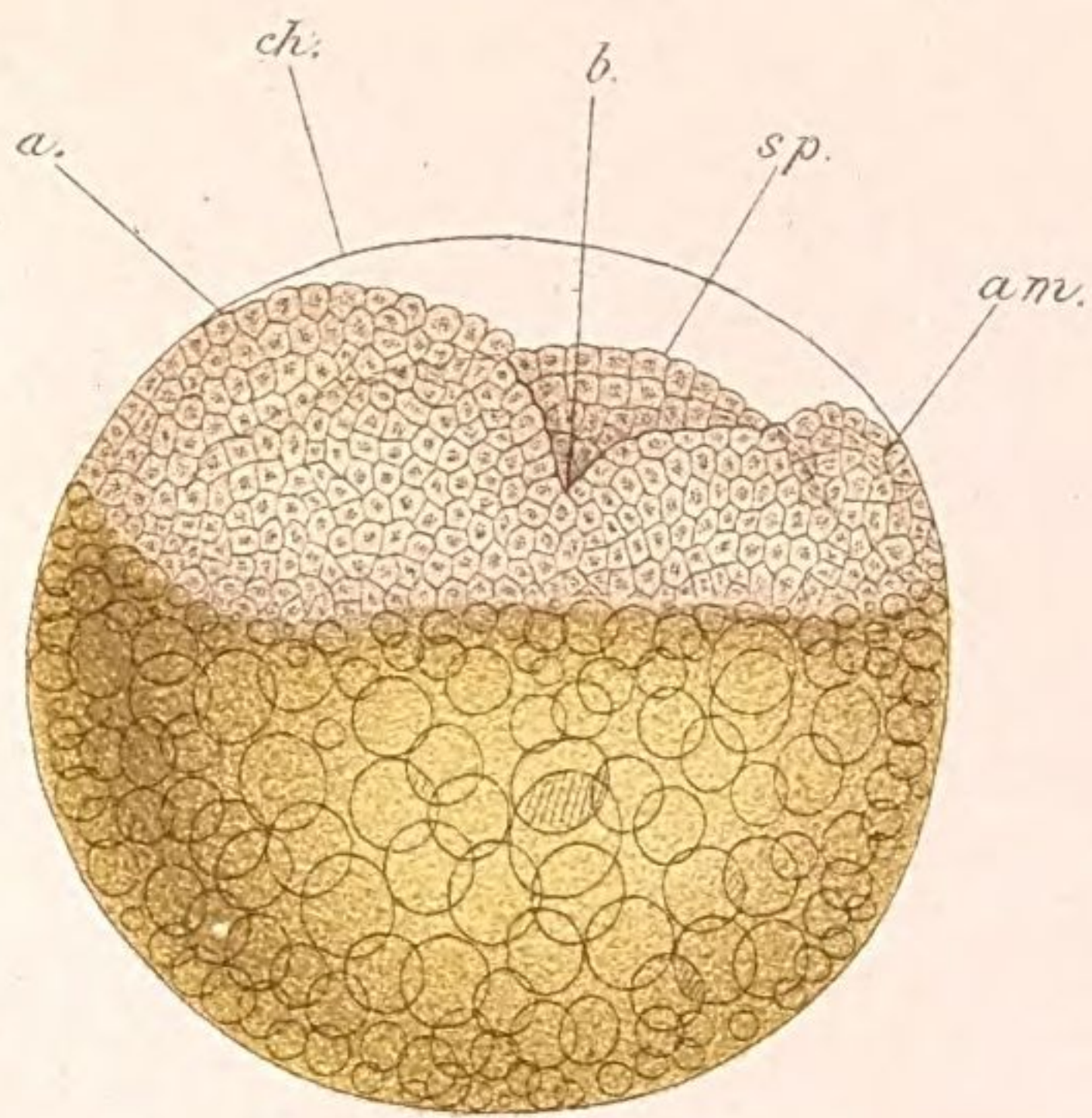
FIG. 38.—A longitudinal section of the same ganglia, showing the longitudinal and double cross commissures.

FIG. 39.—Longitudinal section of the dorsal organ of an embryo shown in Pl. XXXVIA, fig. 15.

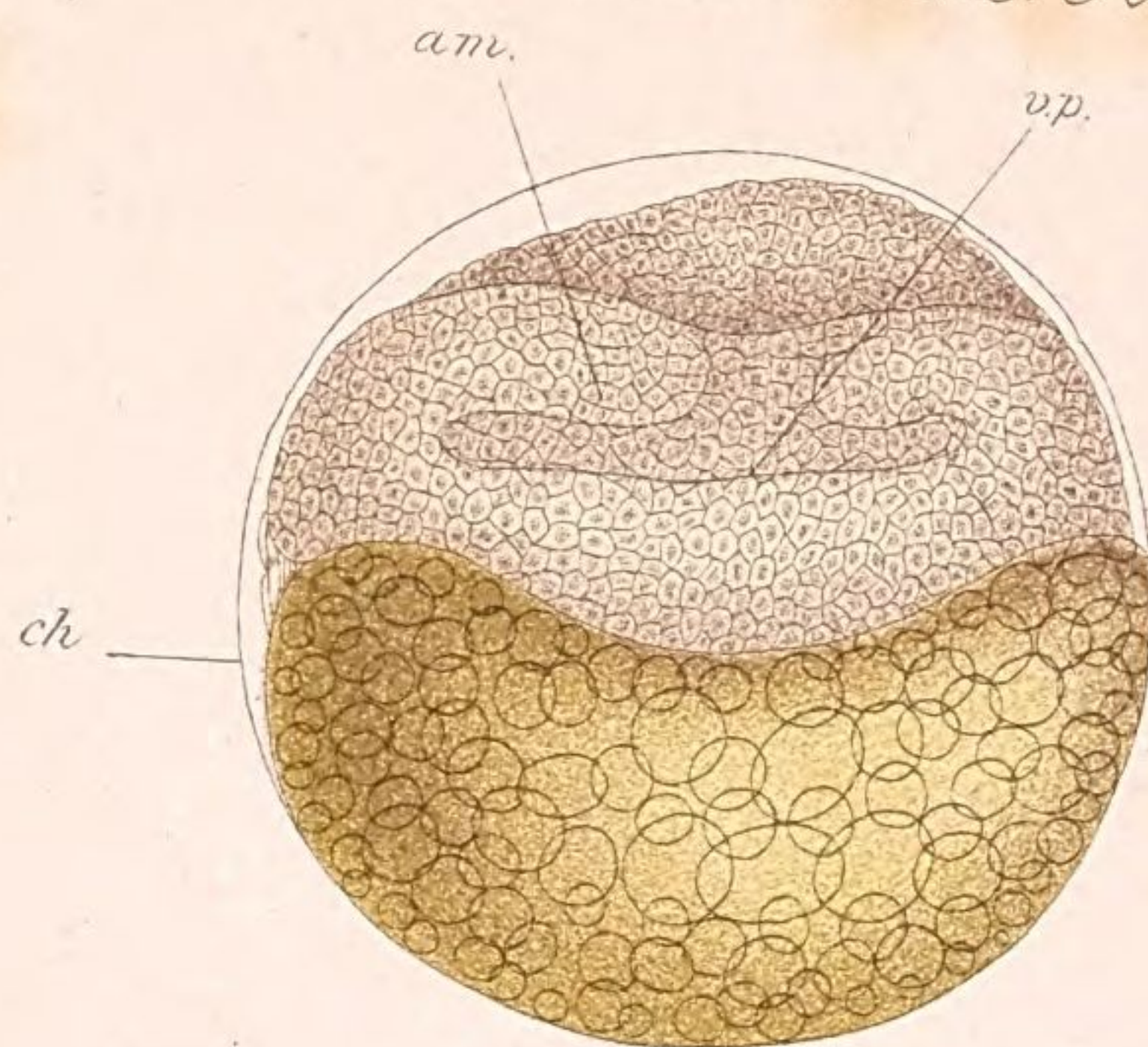
FIGS. 40 and 42.—Successive stages in the disappearance of the dorsal organ.

FIG. 43.—A section of the brain corresponding to fig. 11, Pl. XXXVIA, showing the infolding which separates the brain into two parts. The plane of the section passes through both antennæ and the point marked *a*.

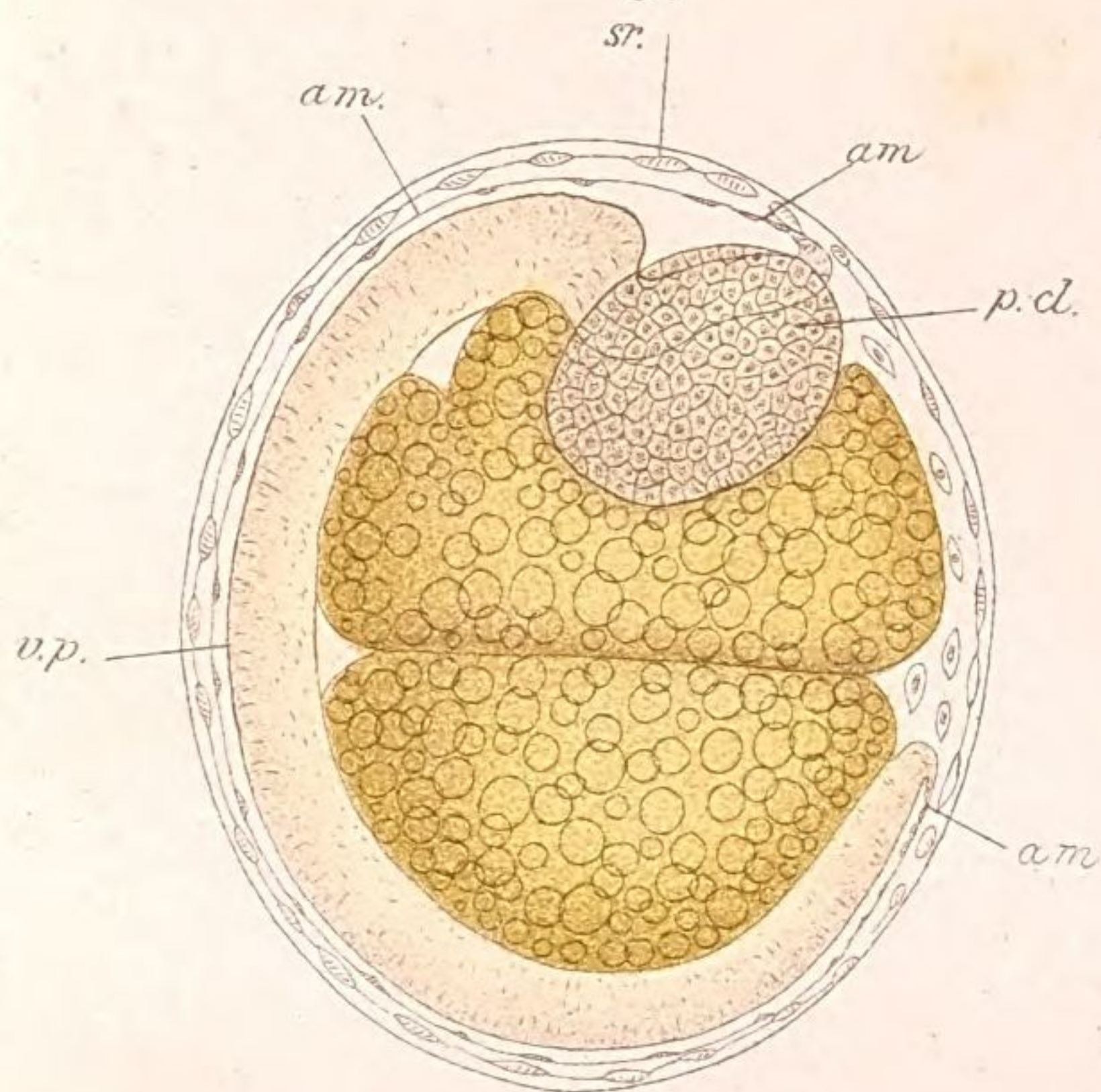




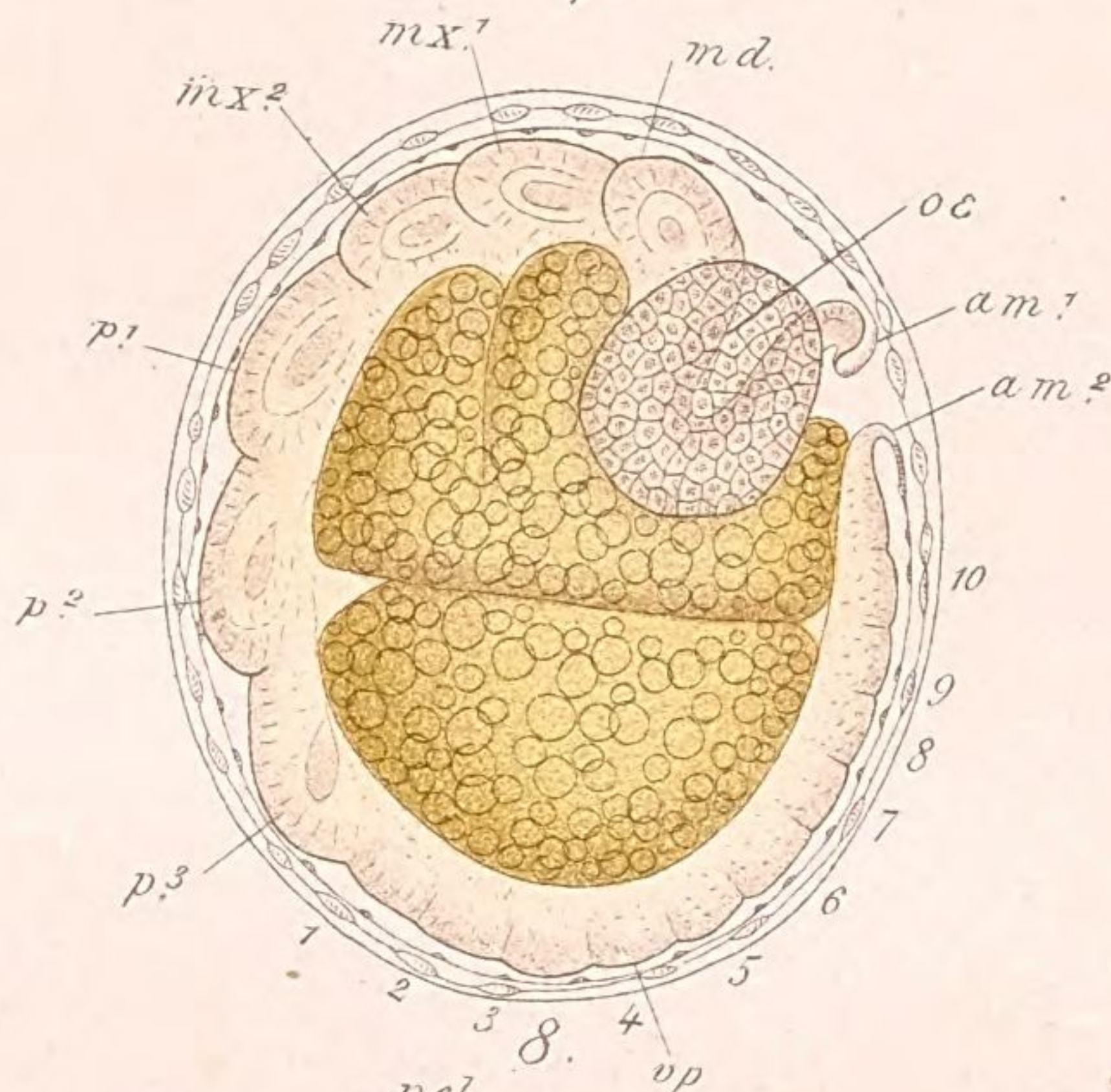
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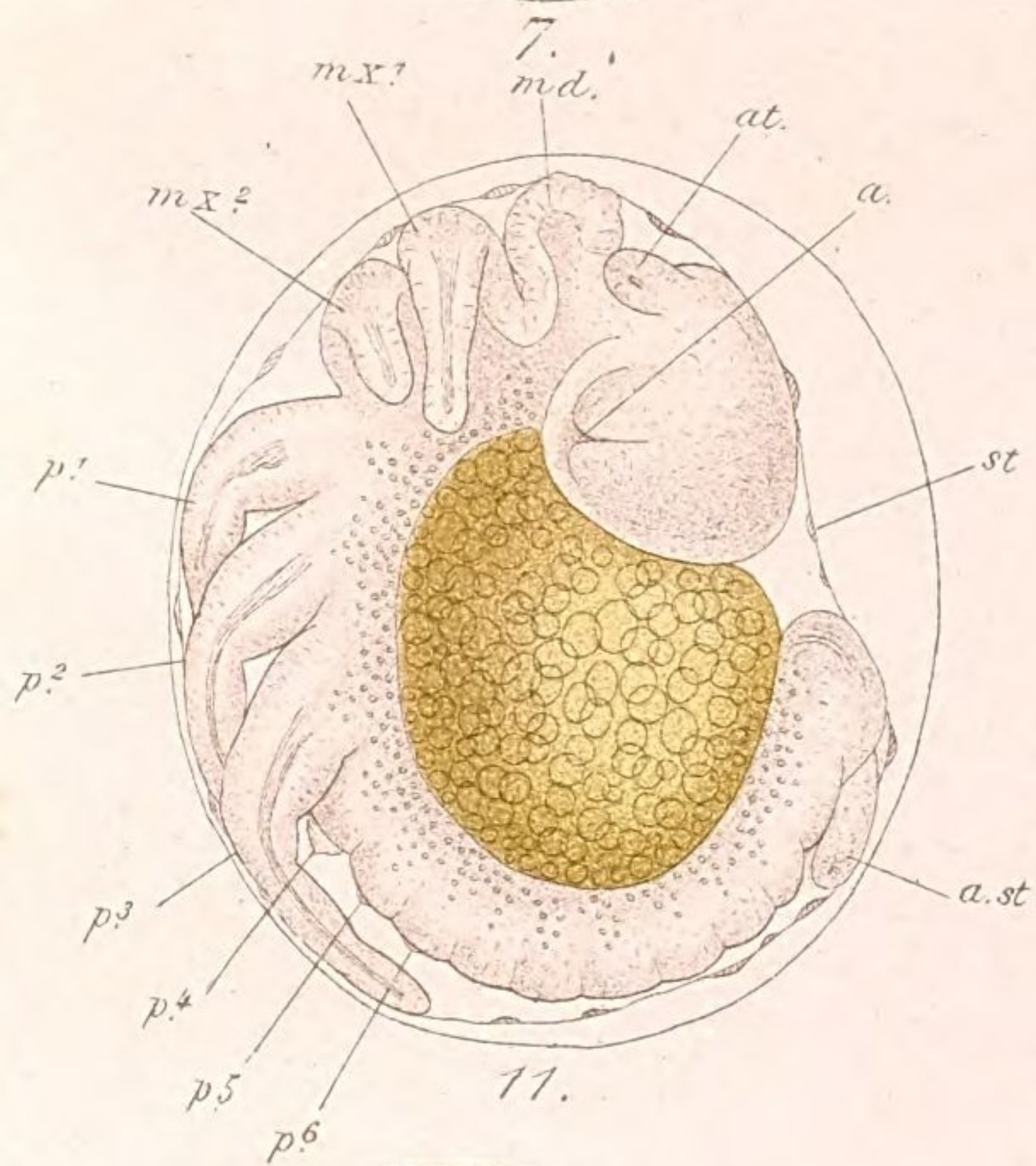
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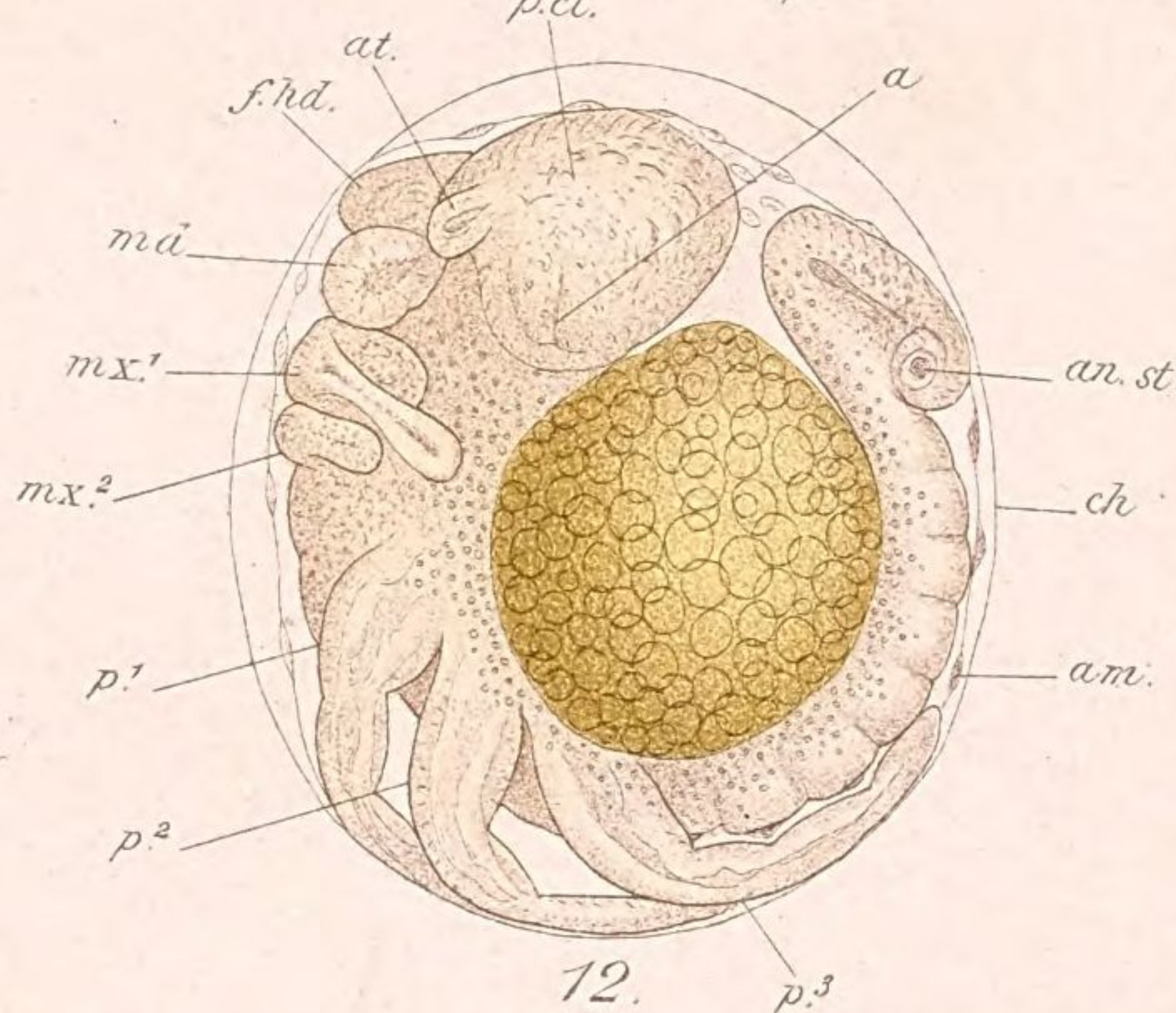
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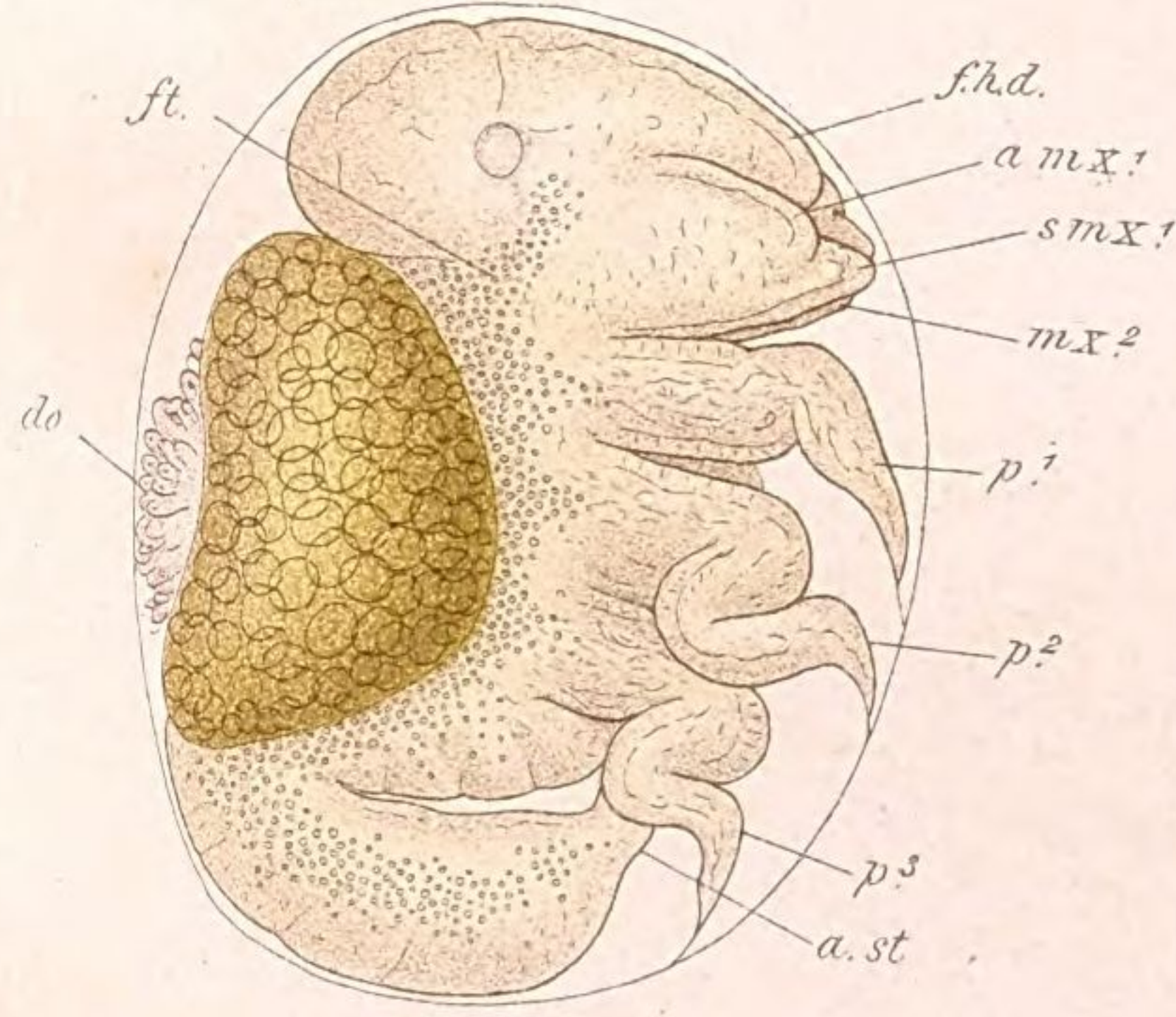
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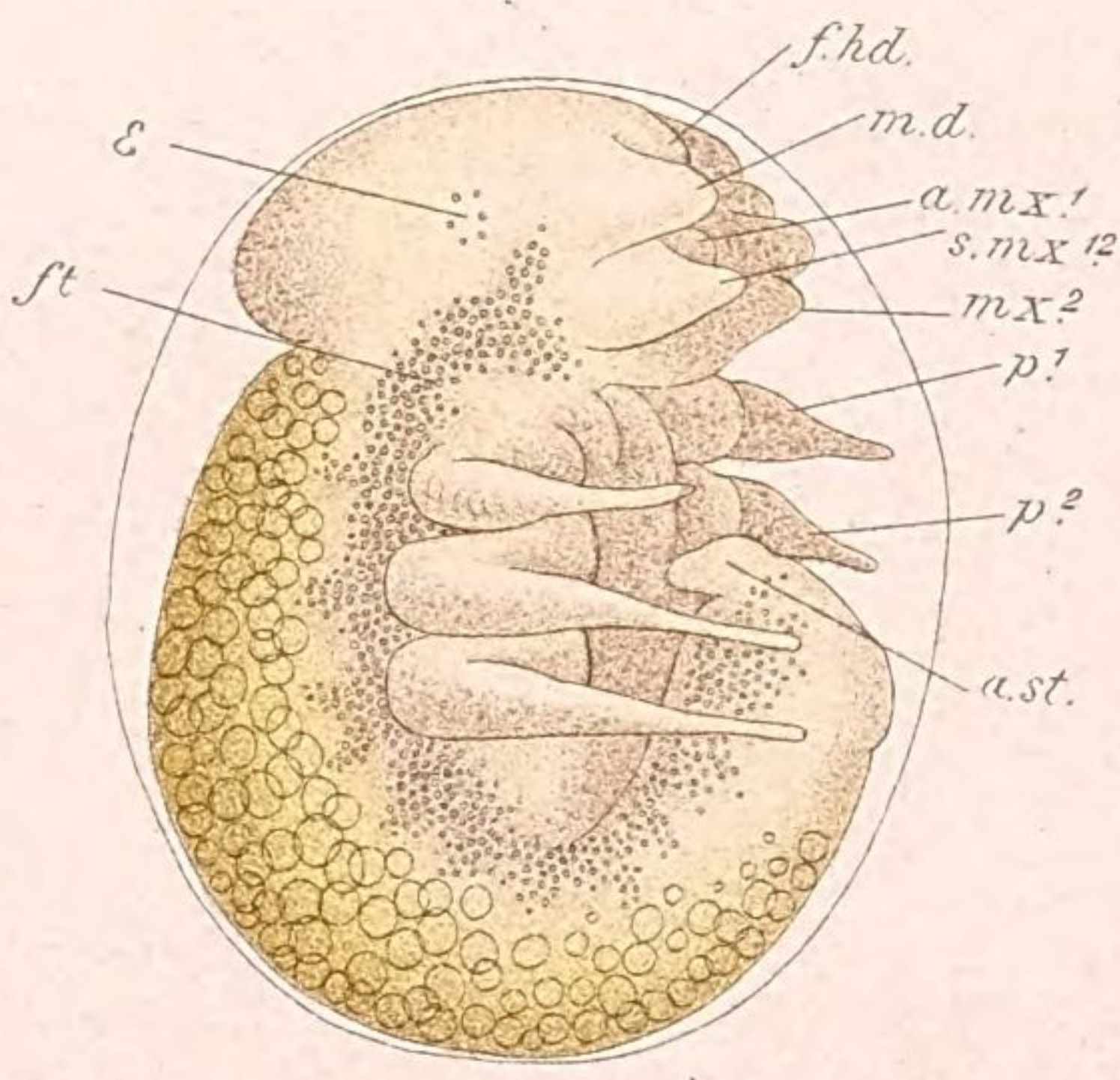
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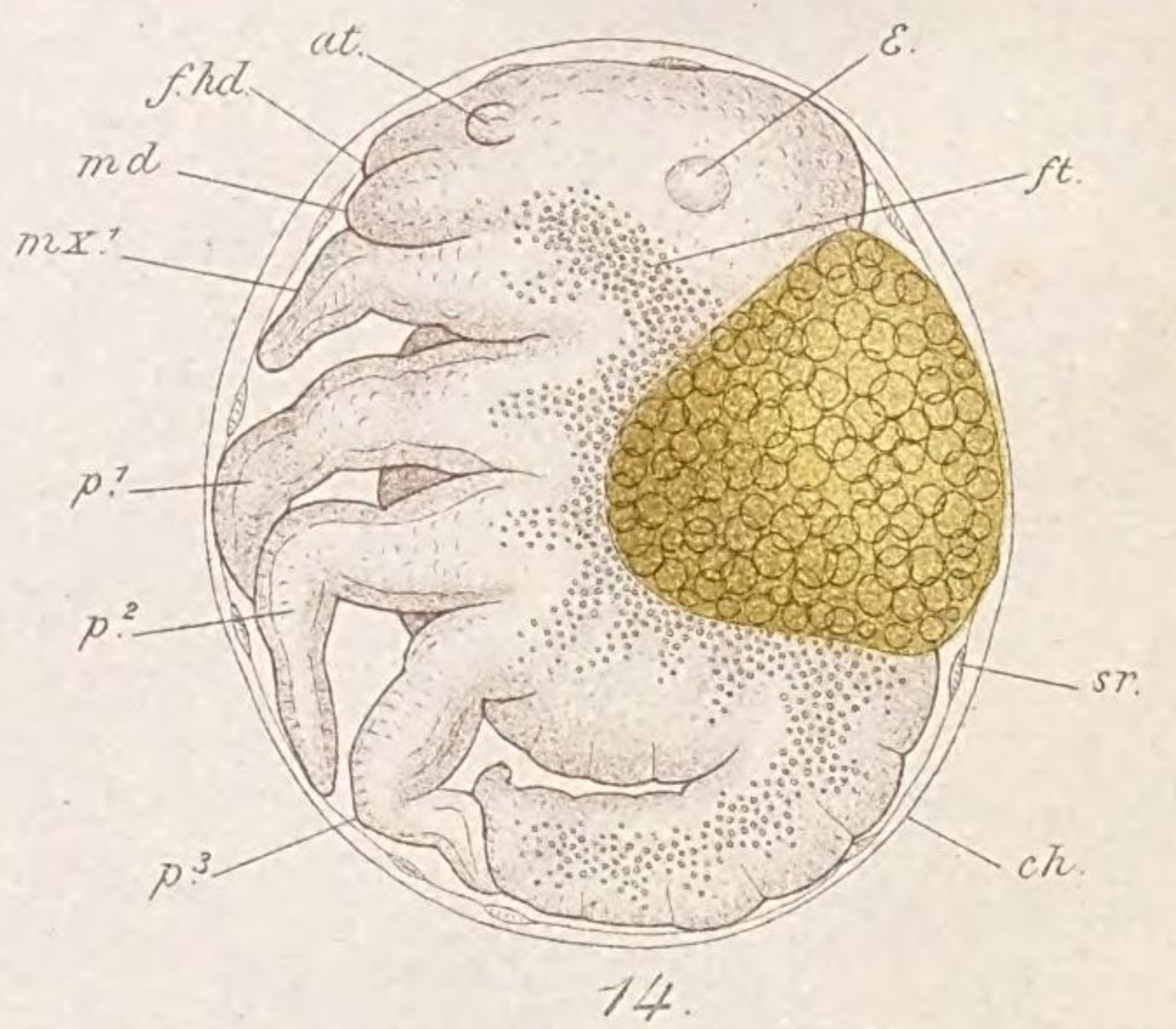
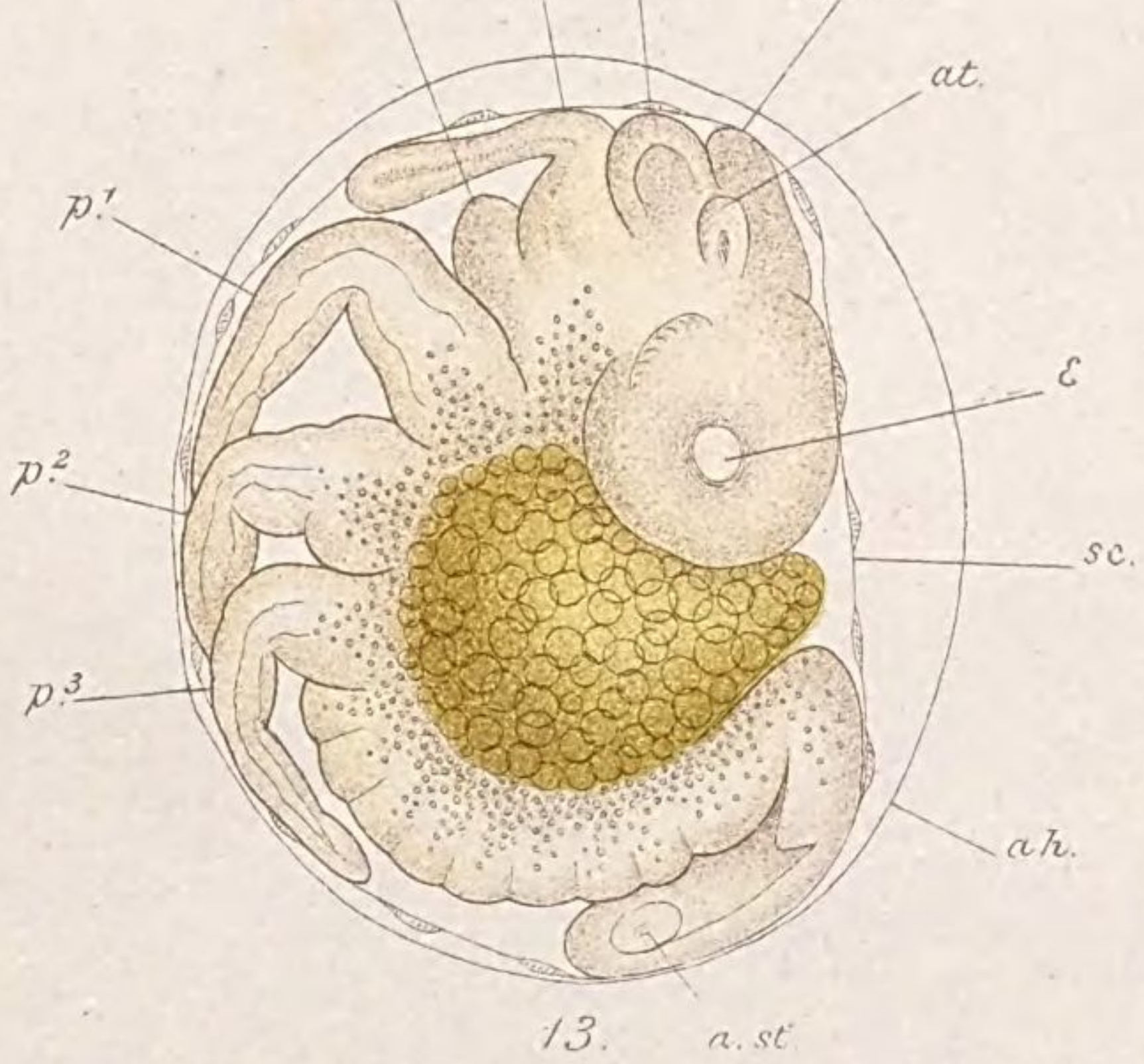
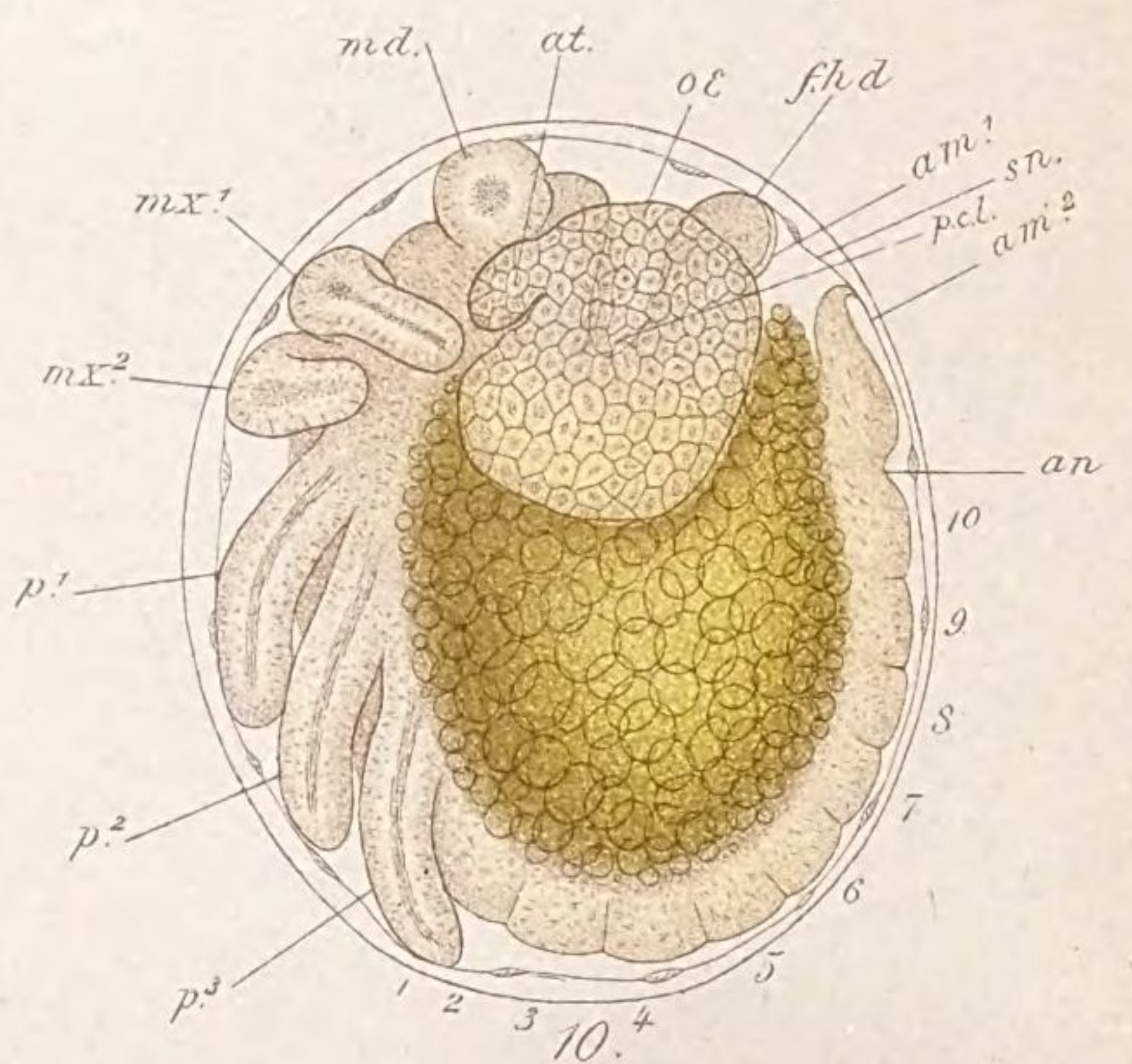
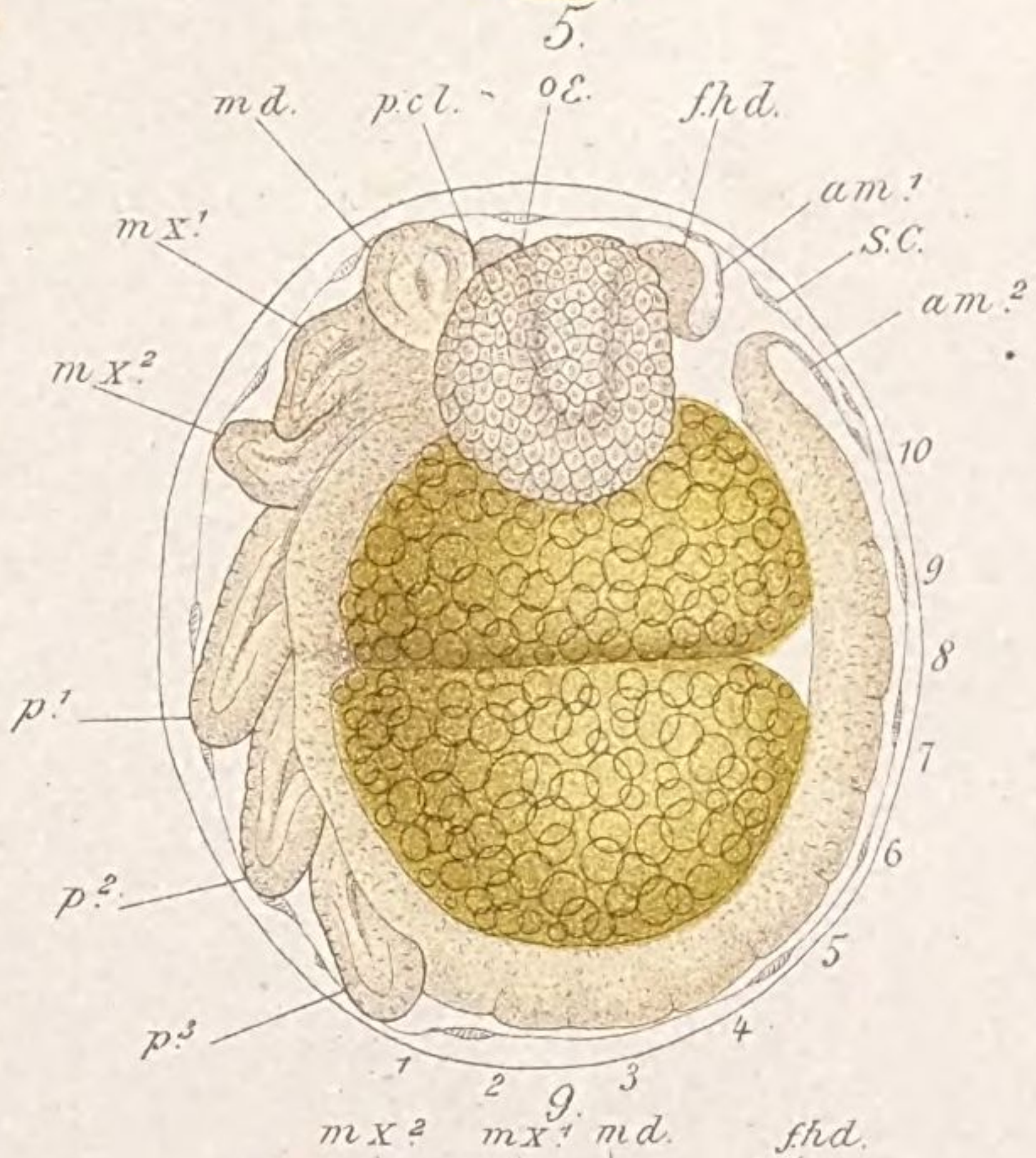
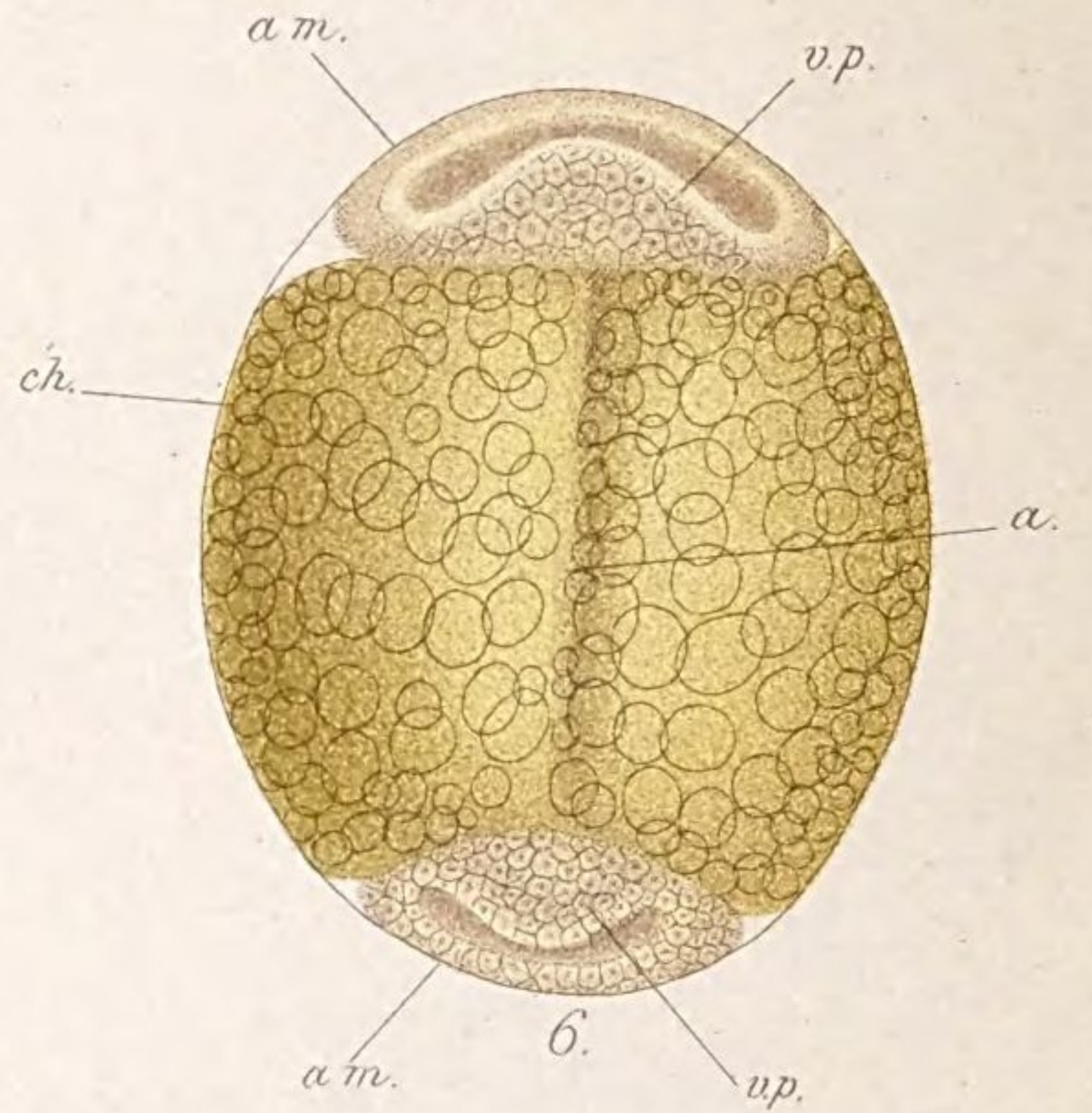
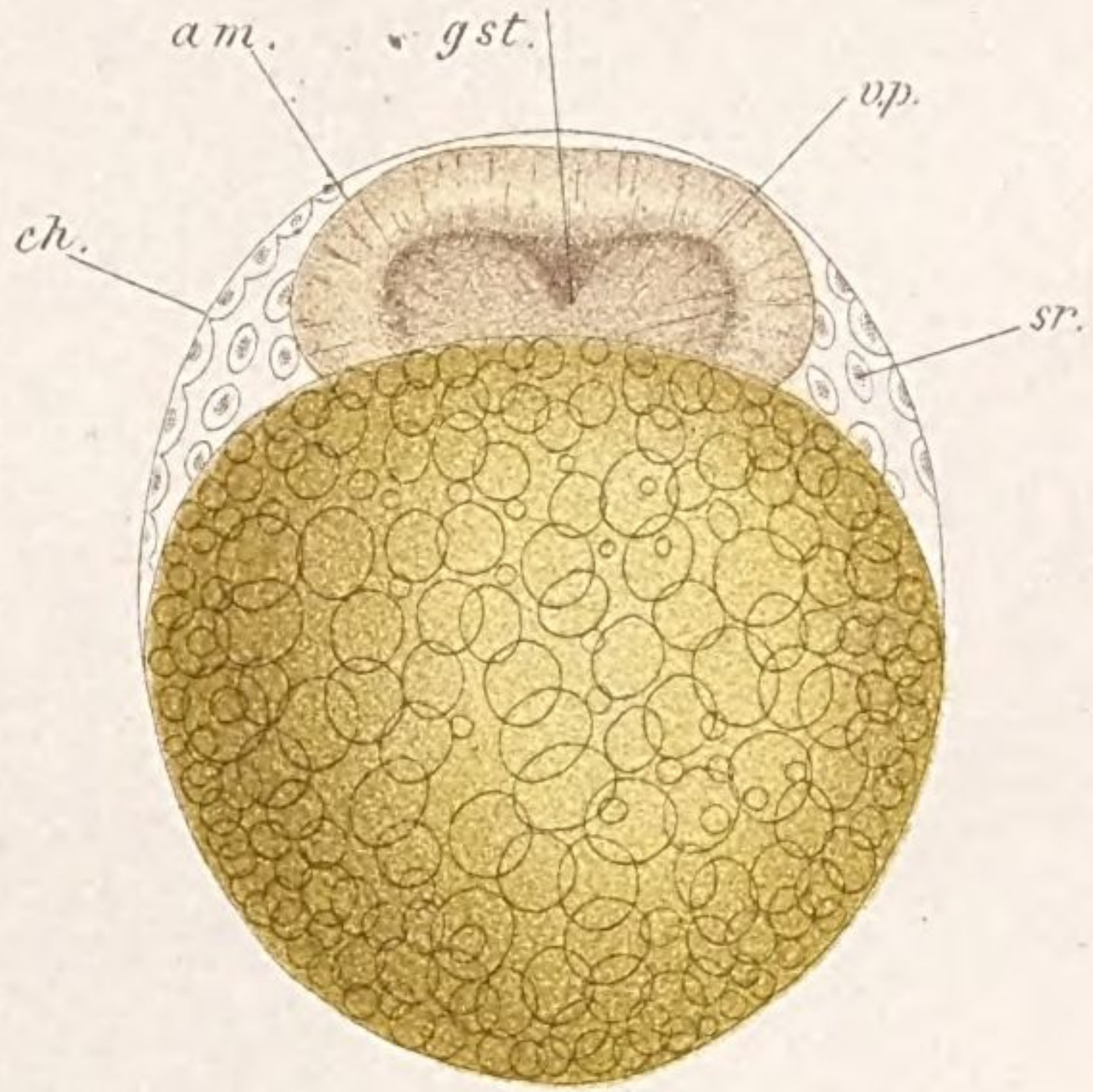
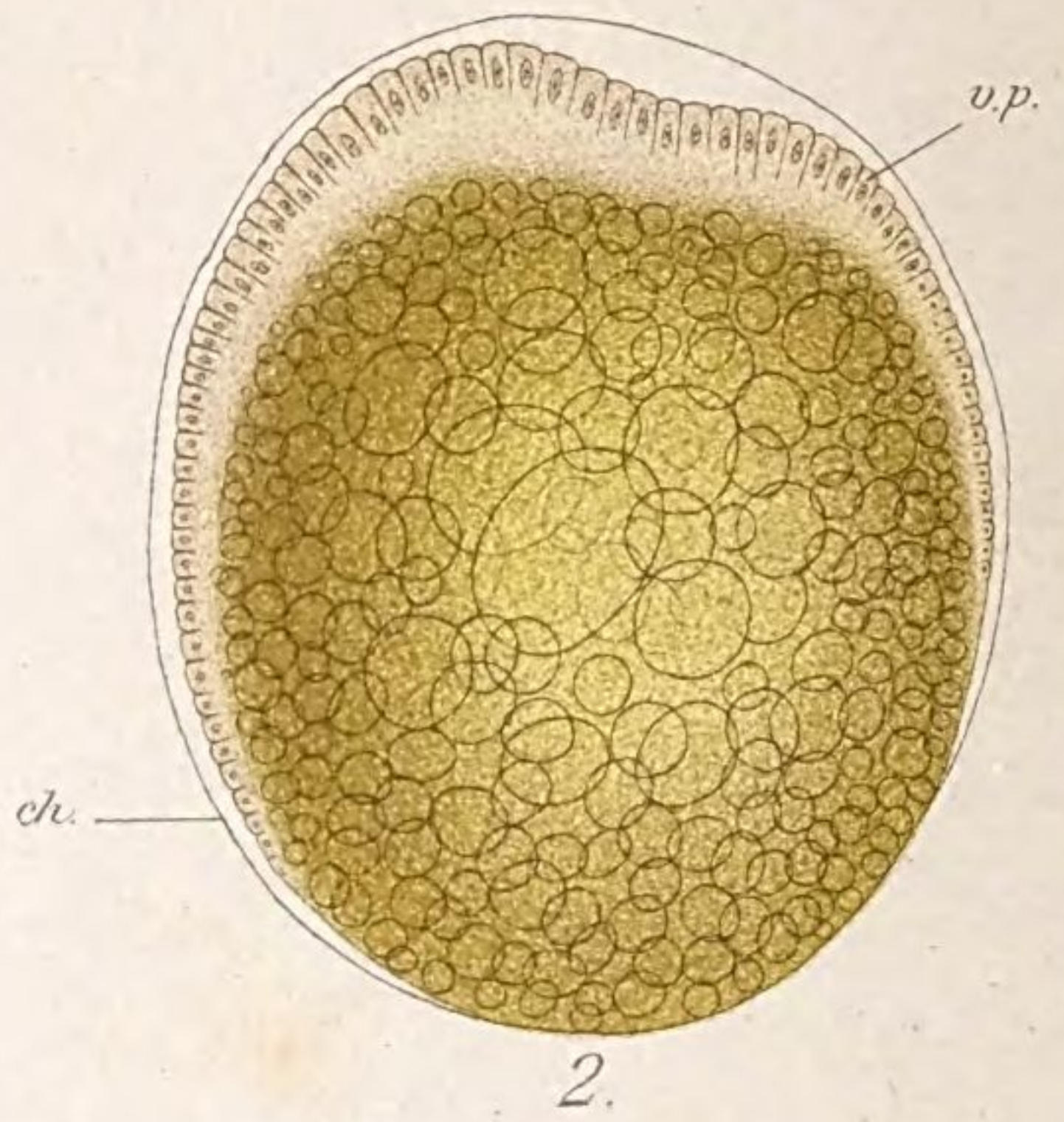
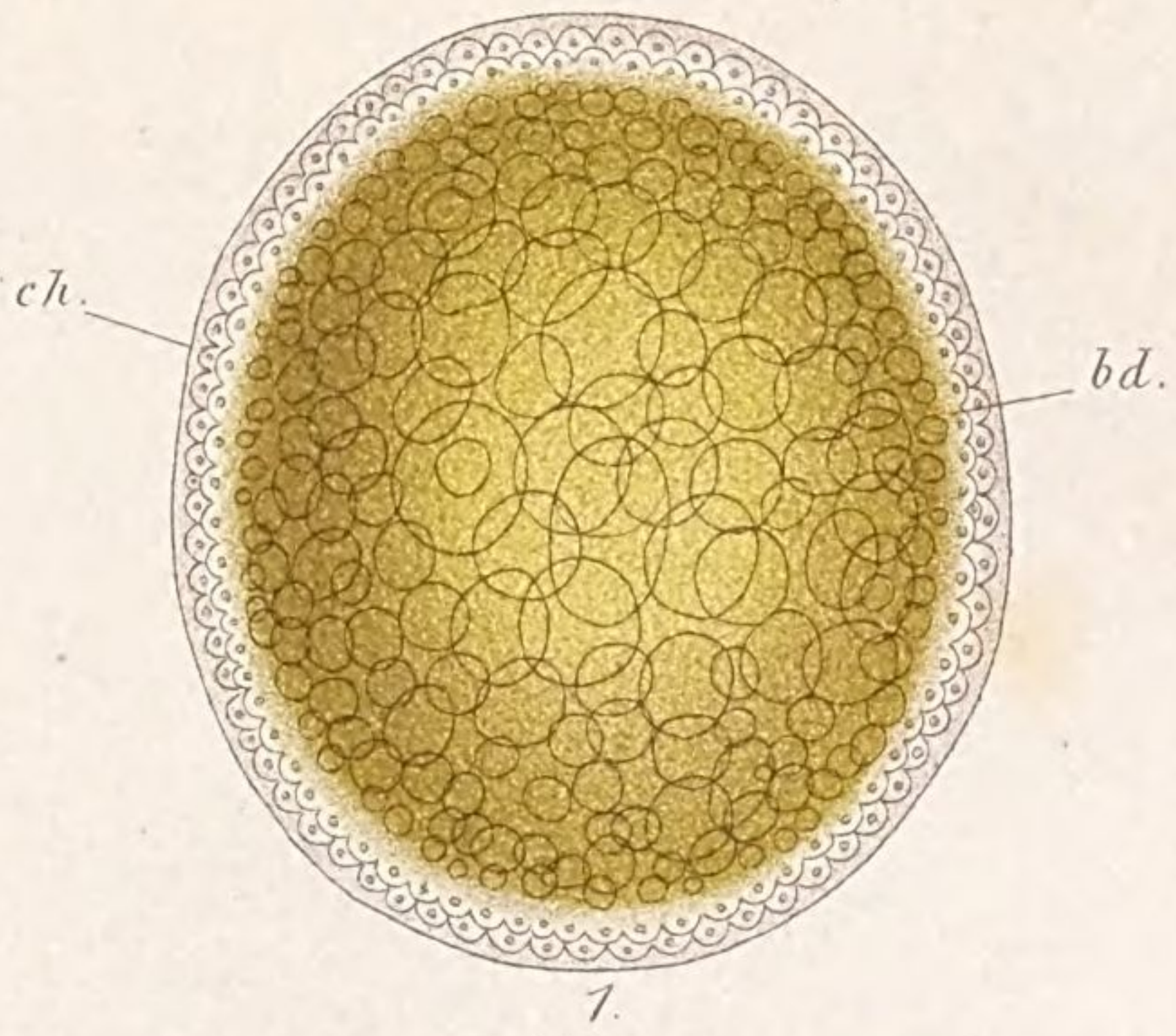
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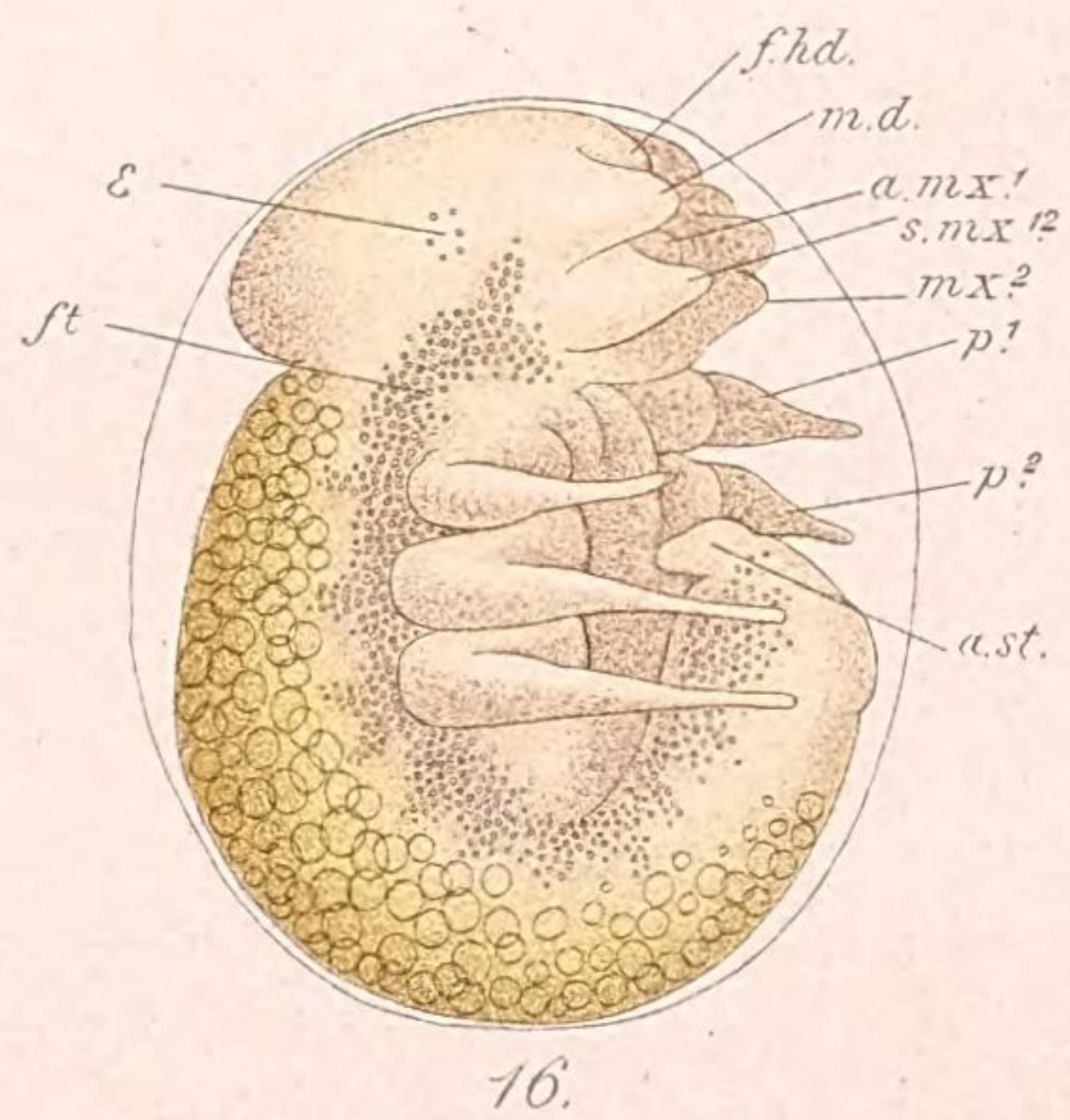
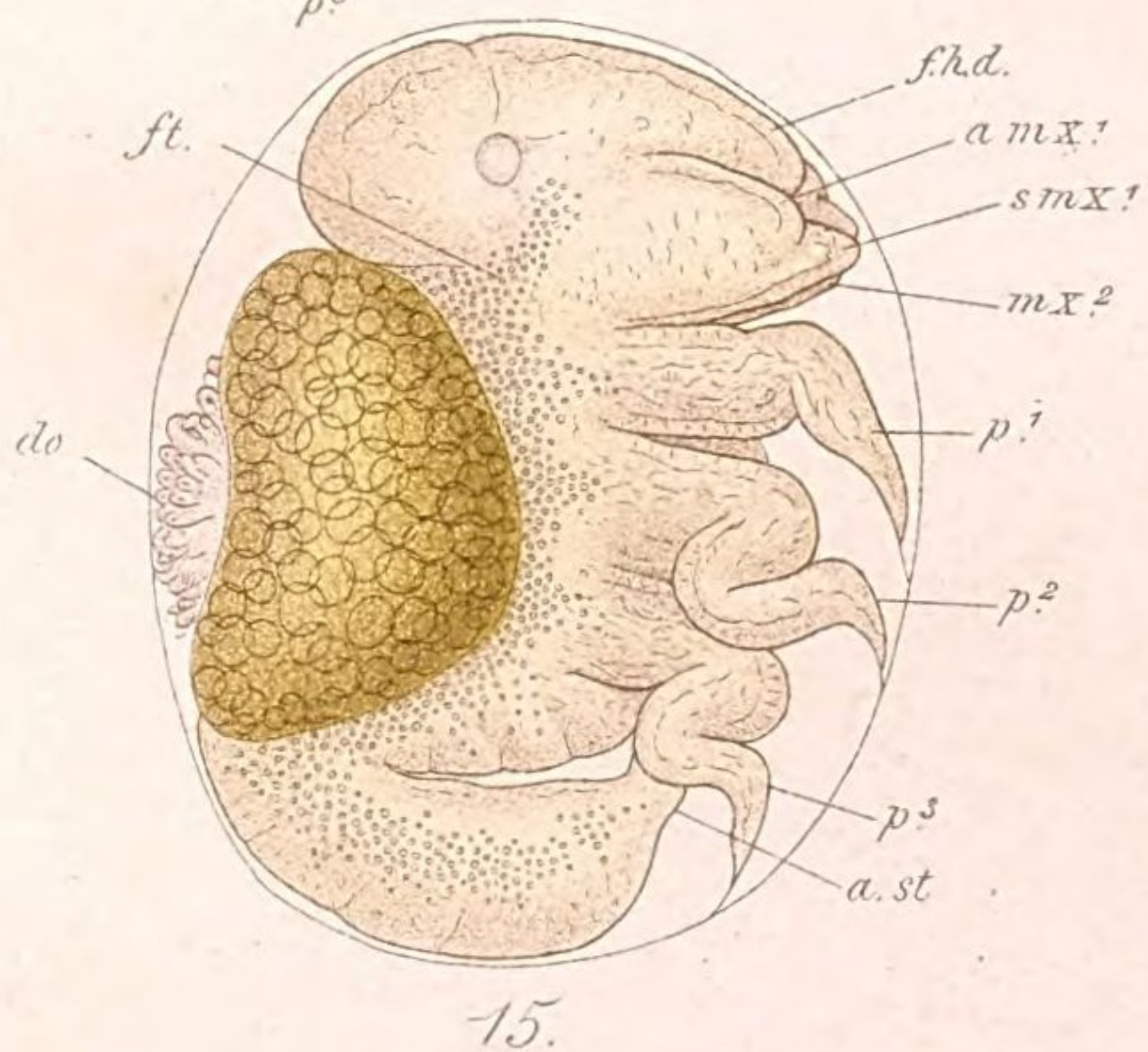
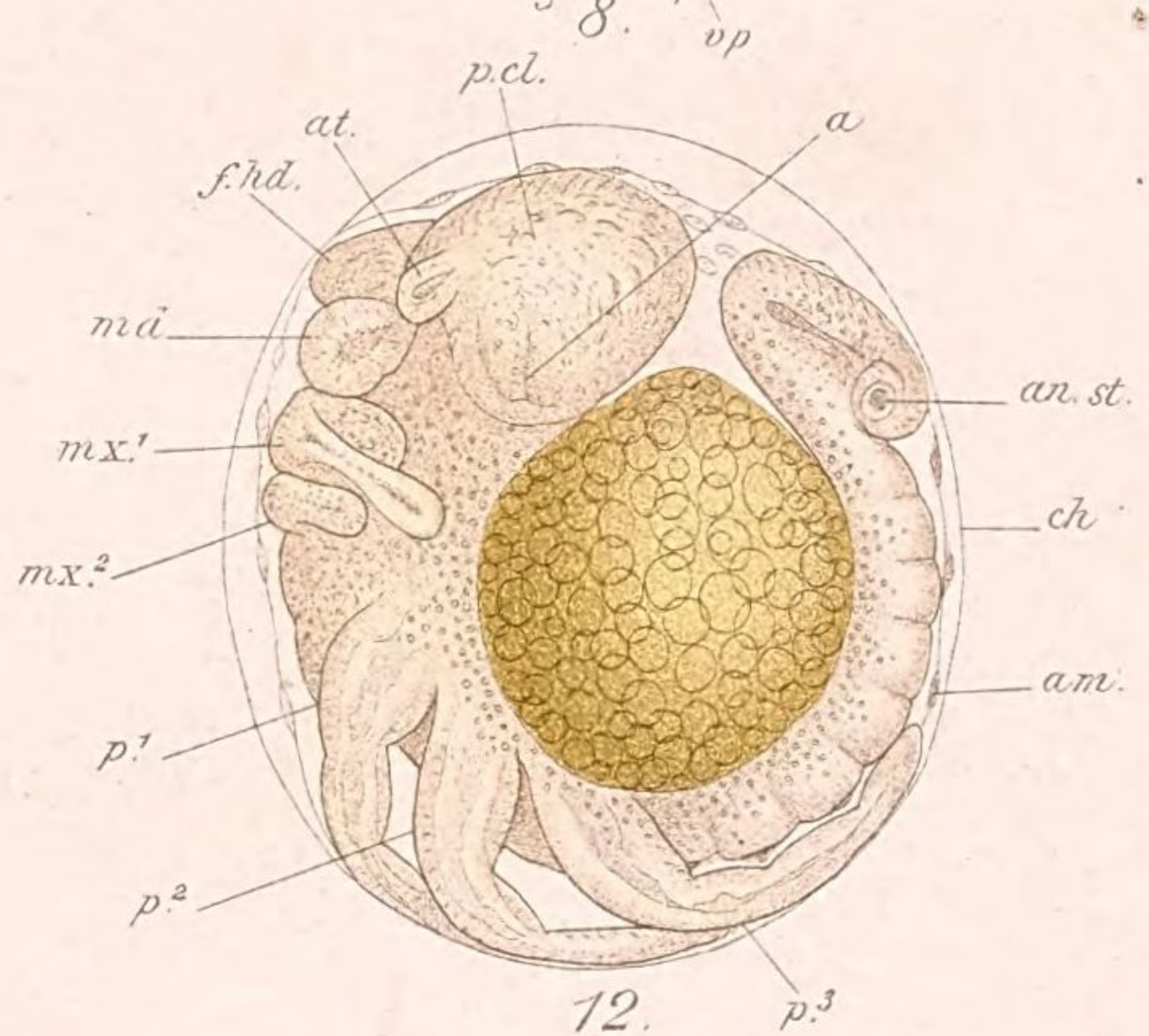
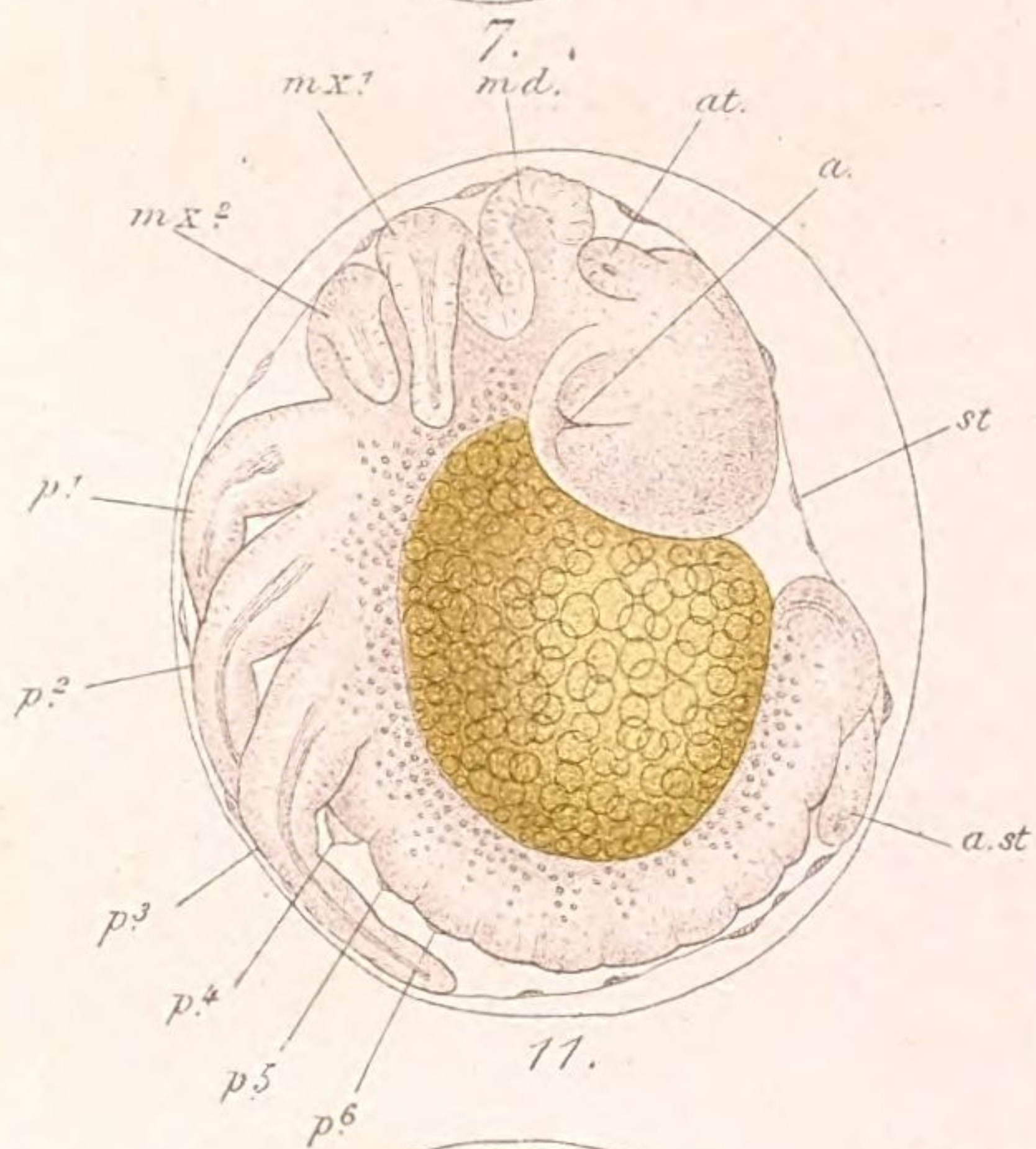
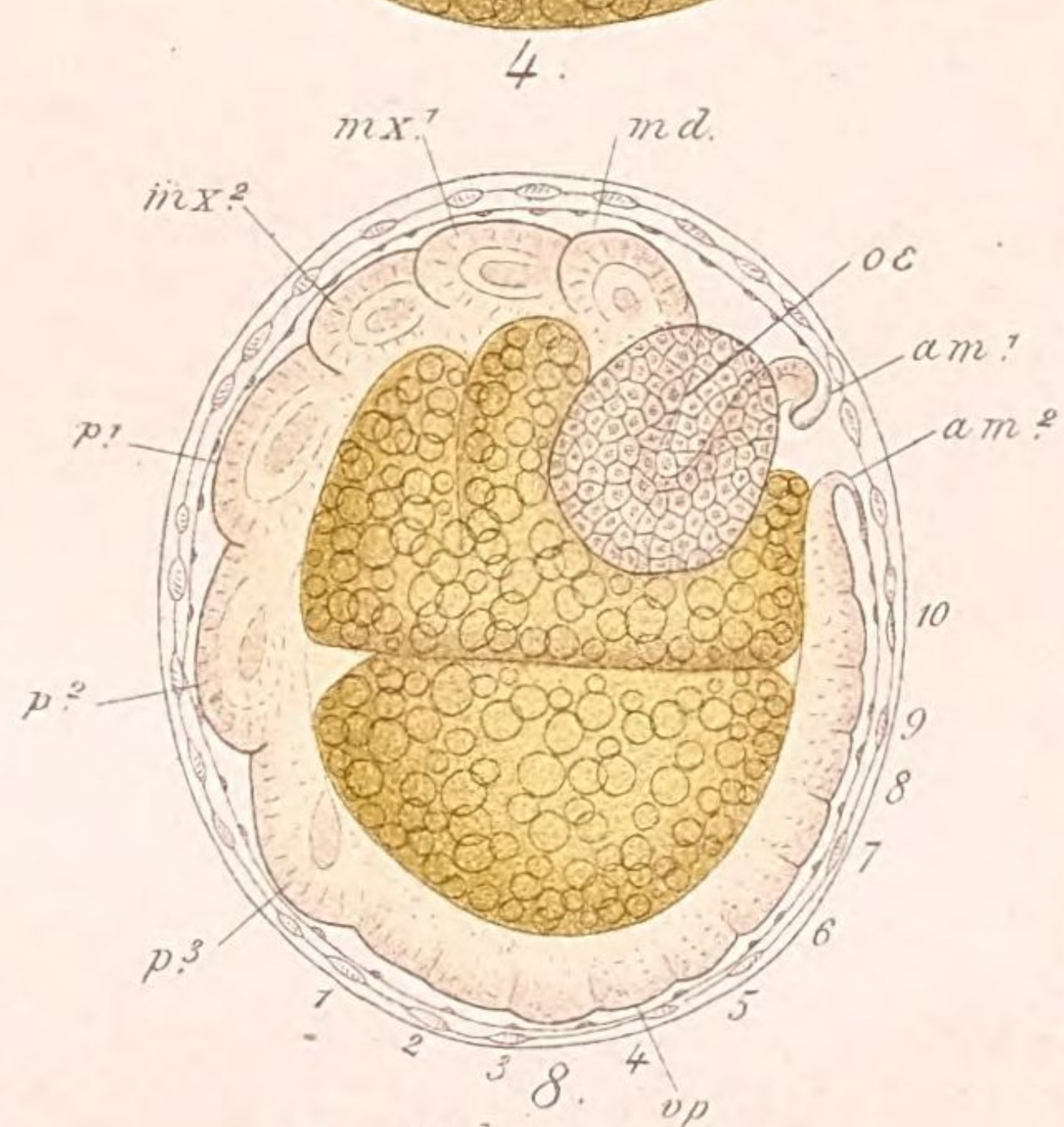
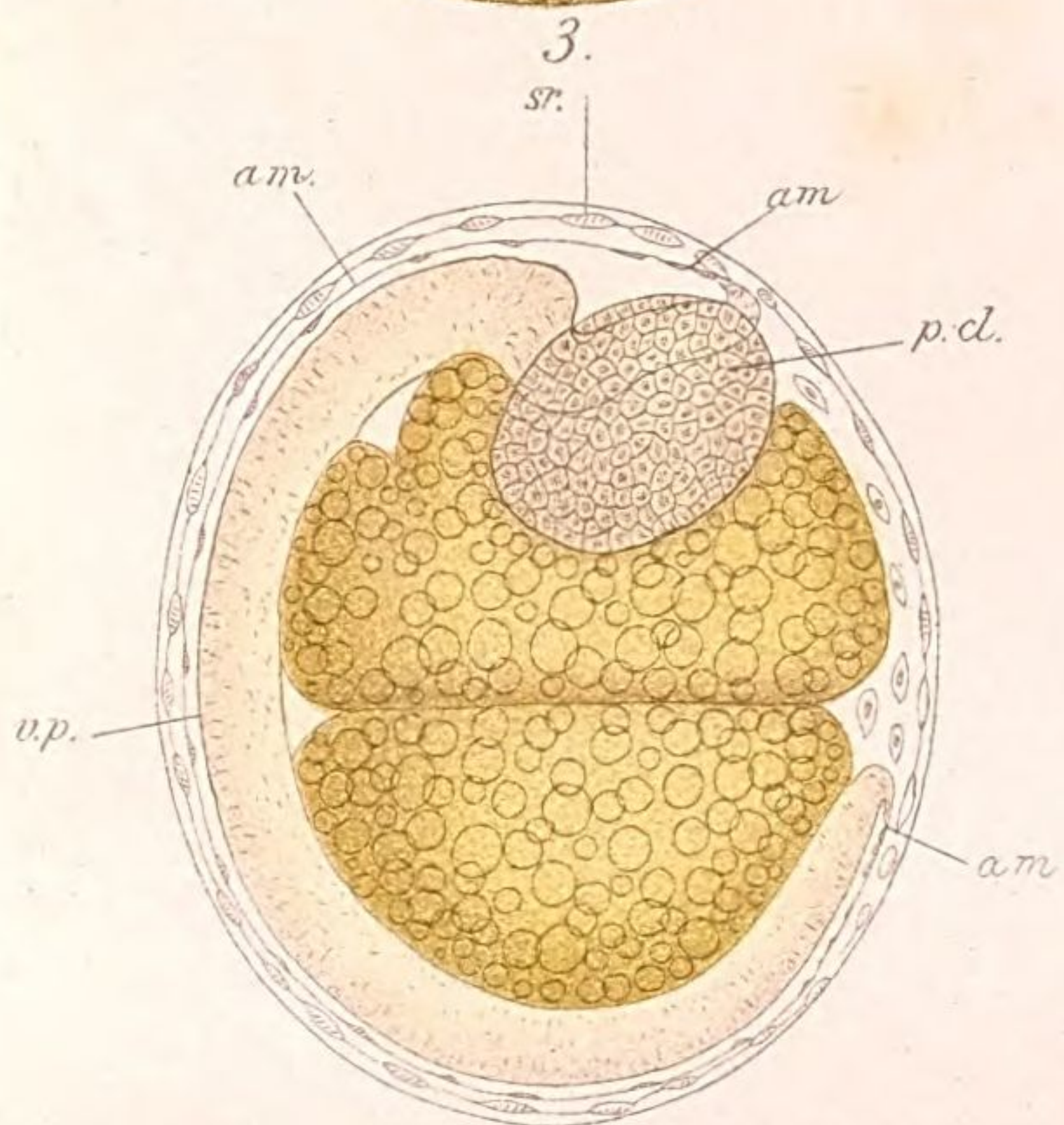
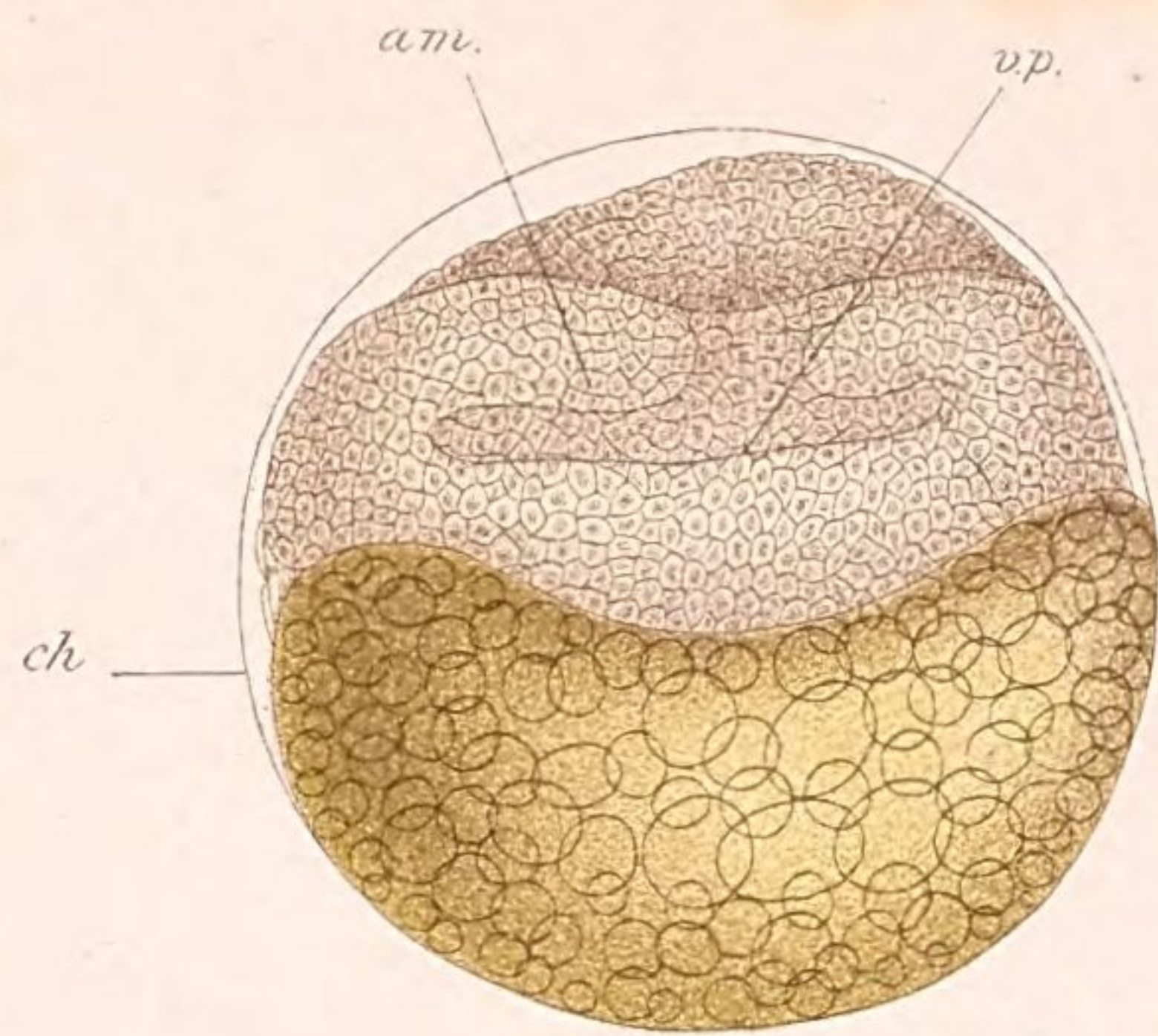
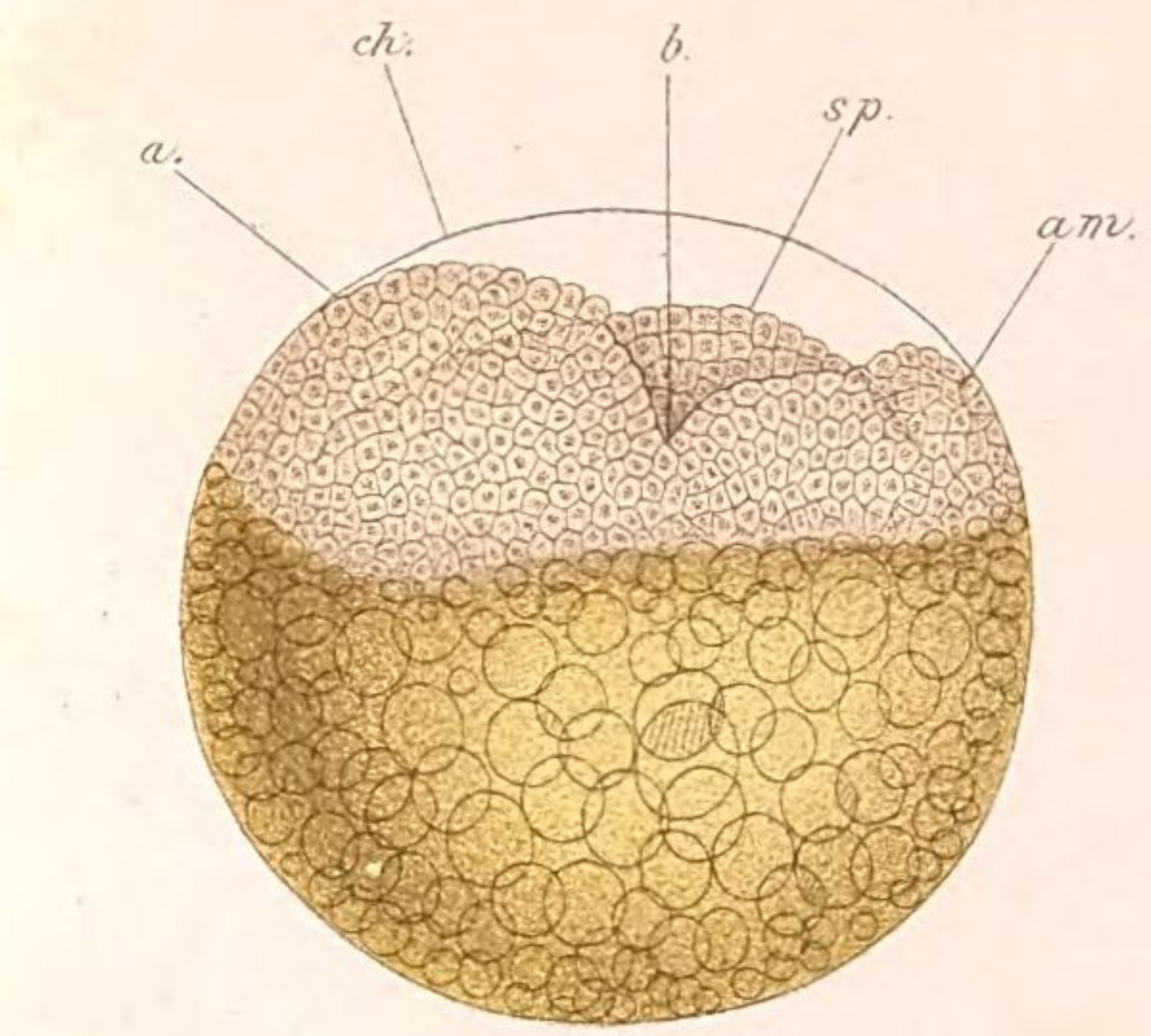


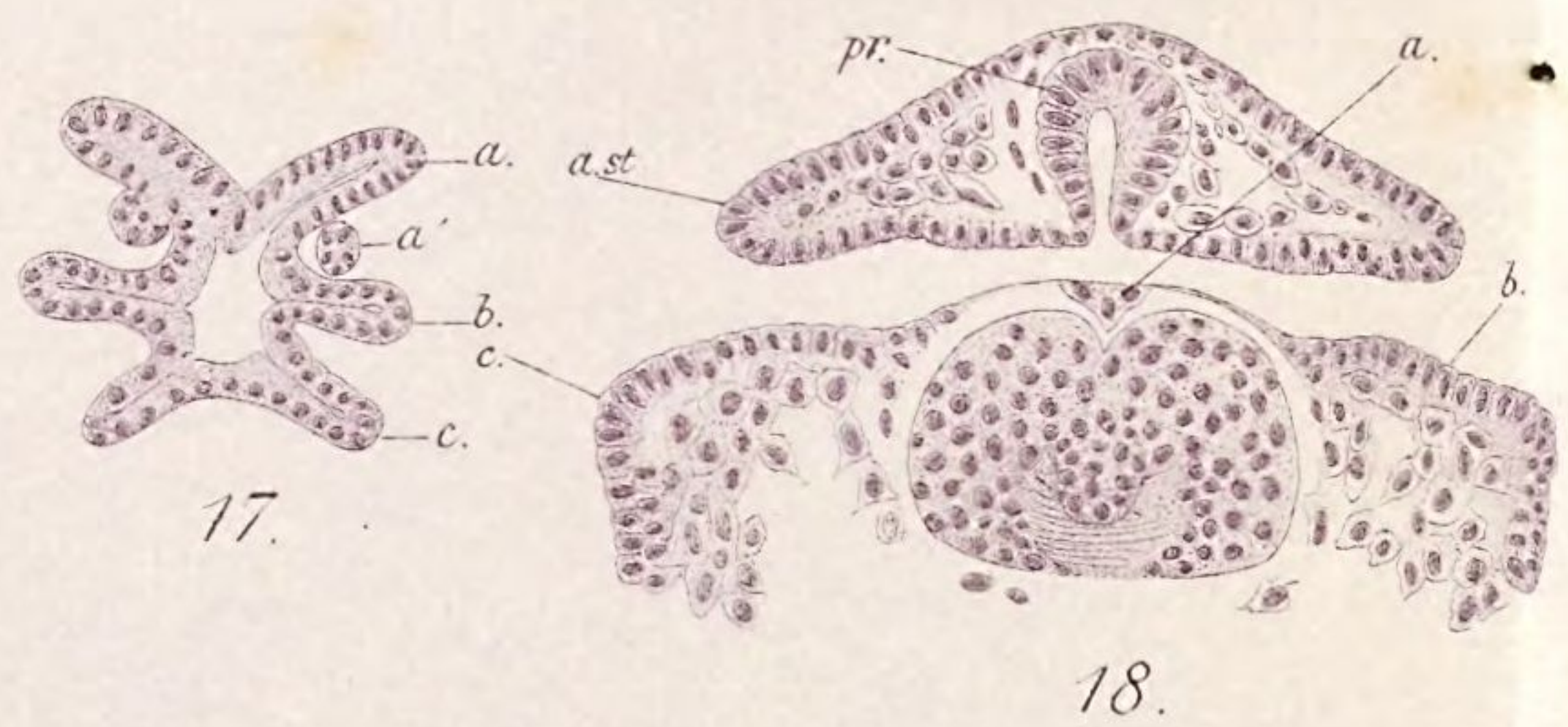
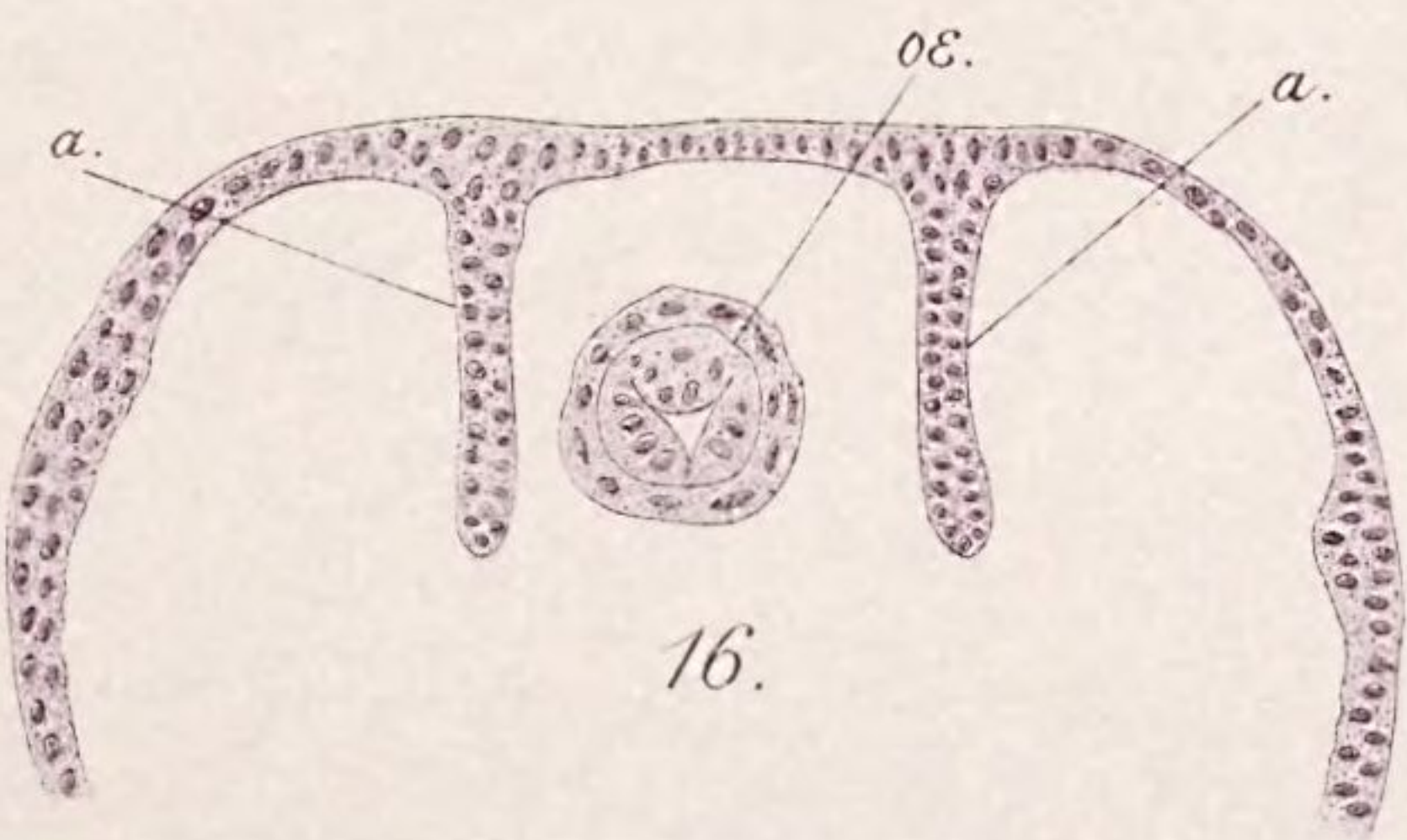
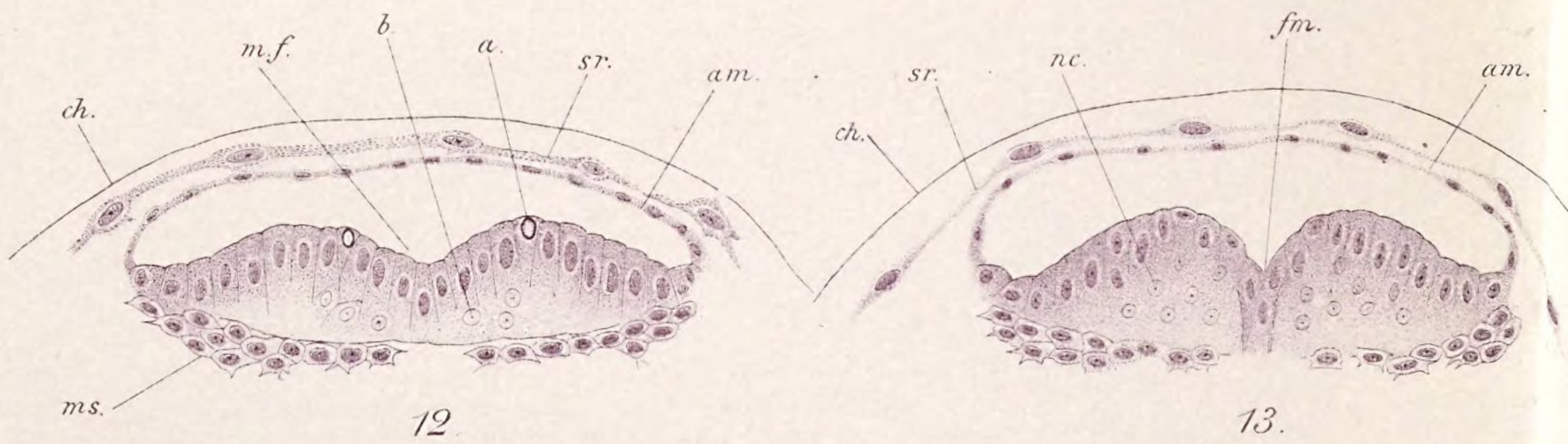
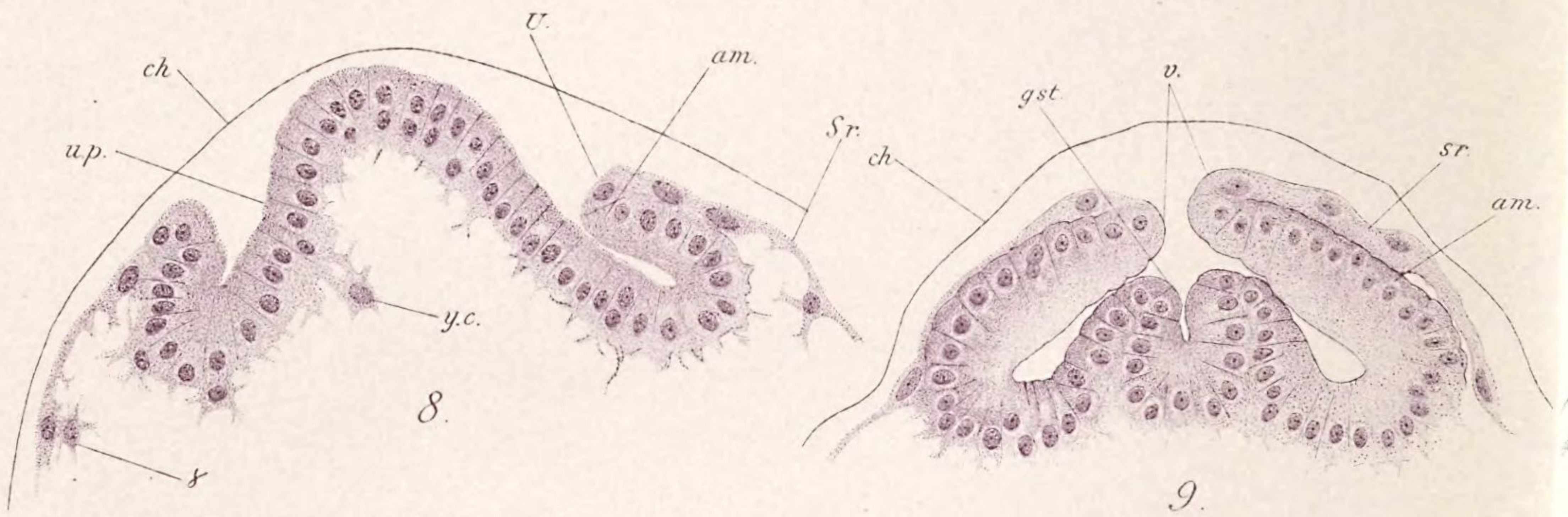
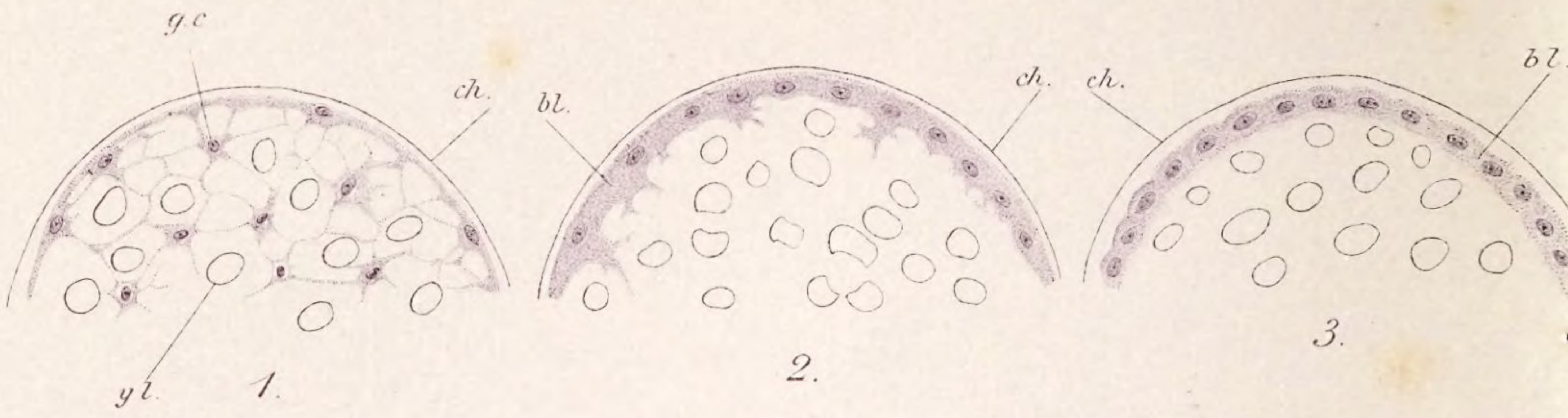
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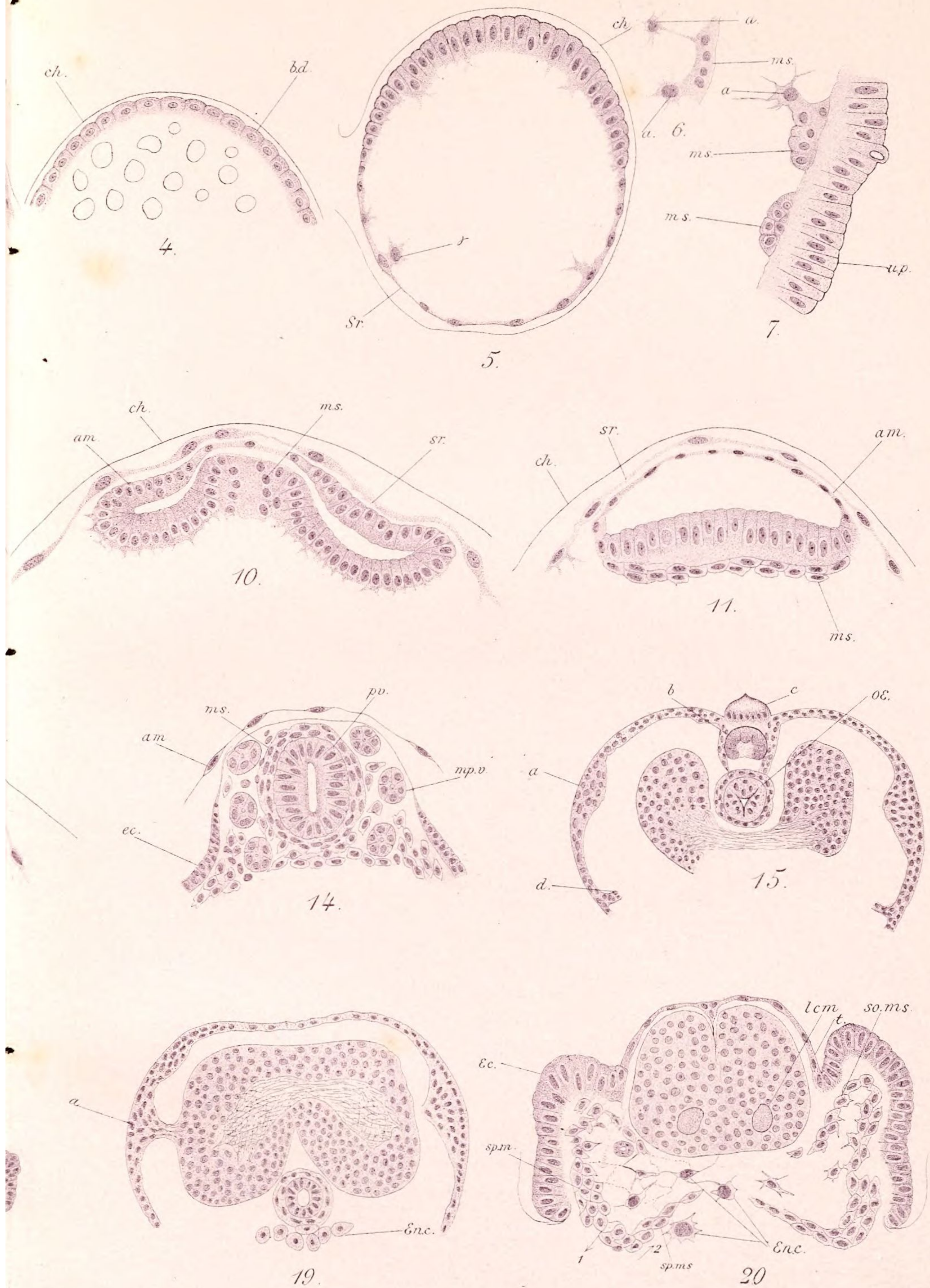


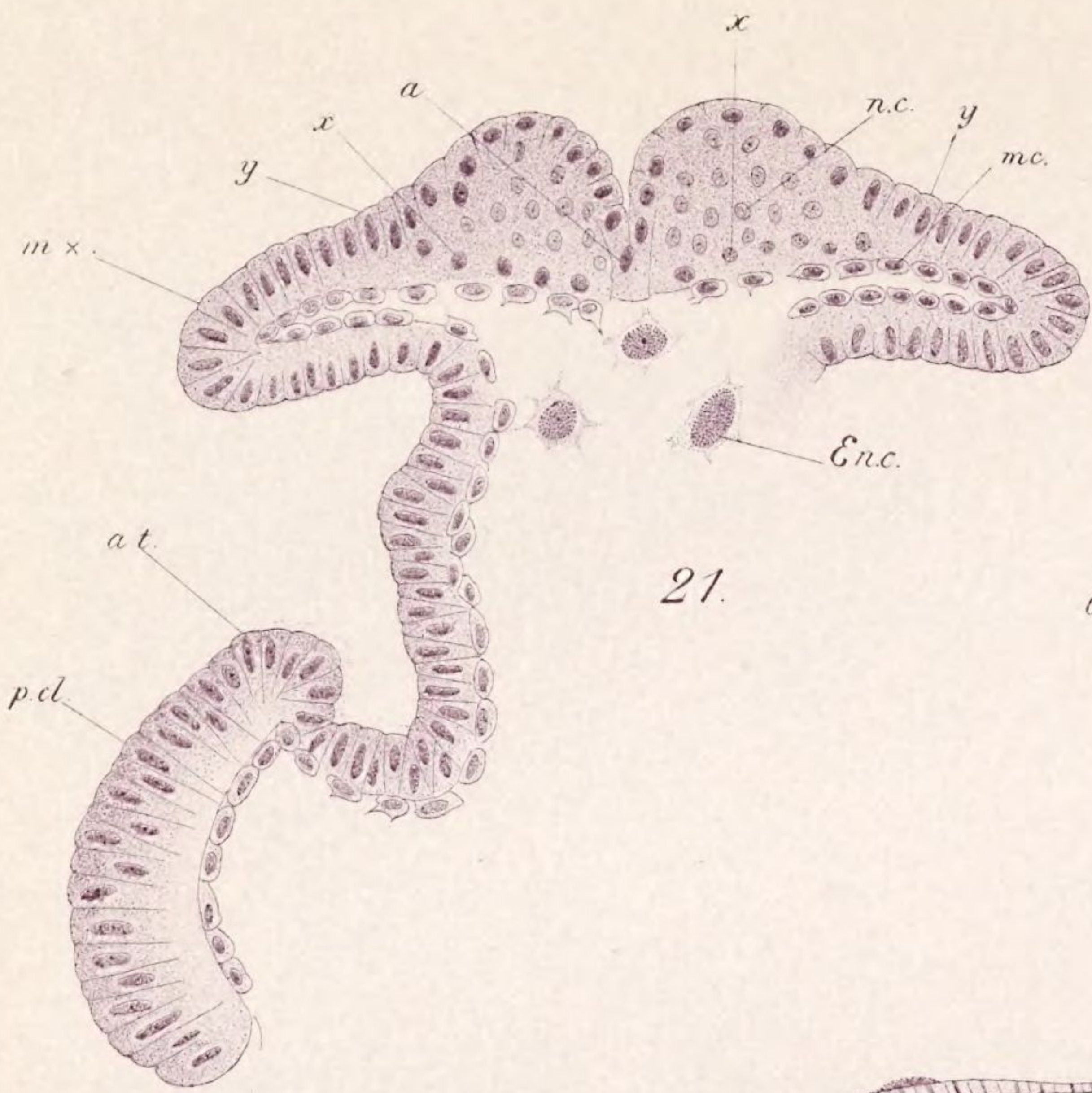
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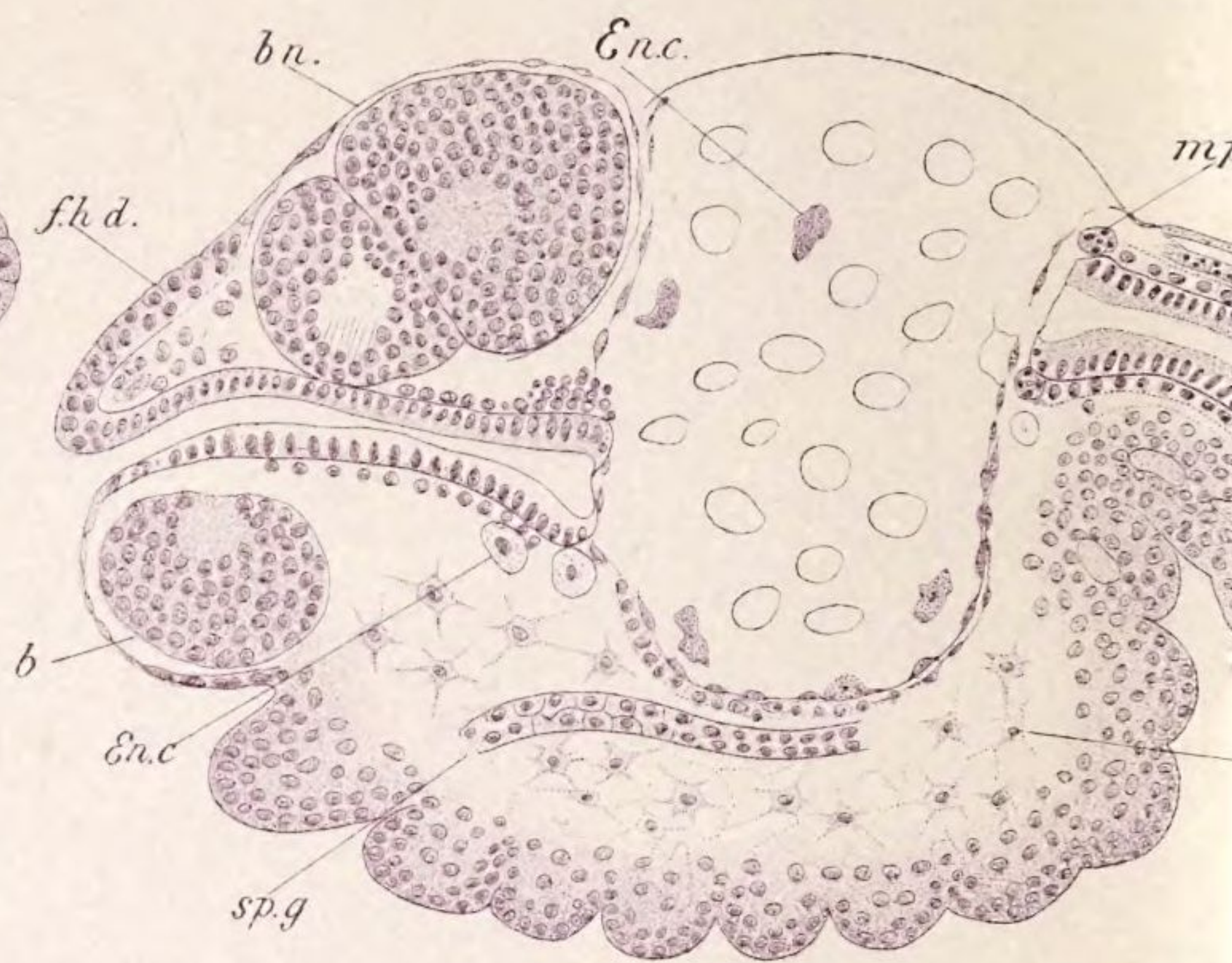




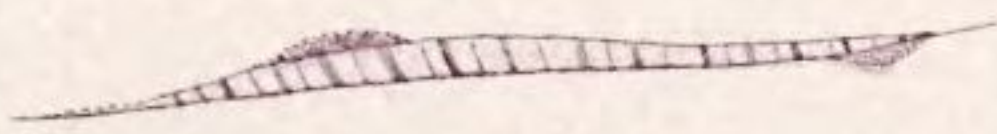




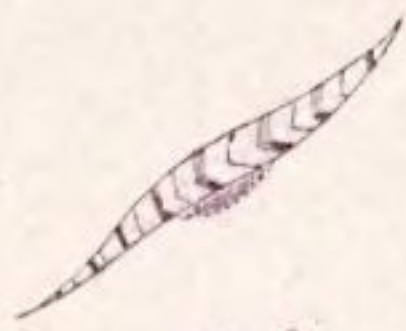
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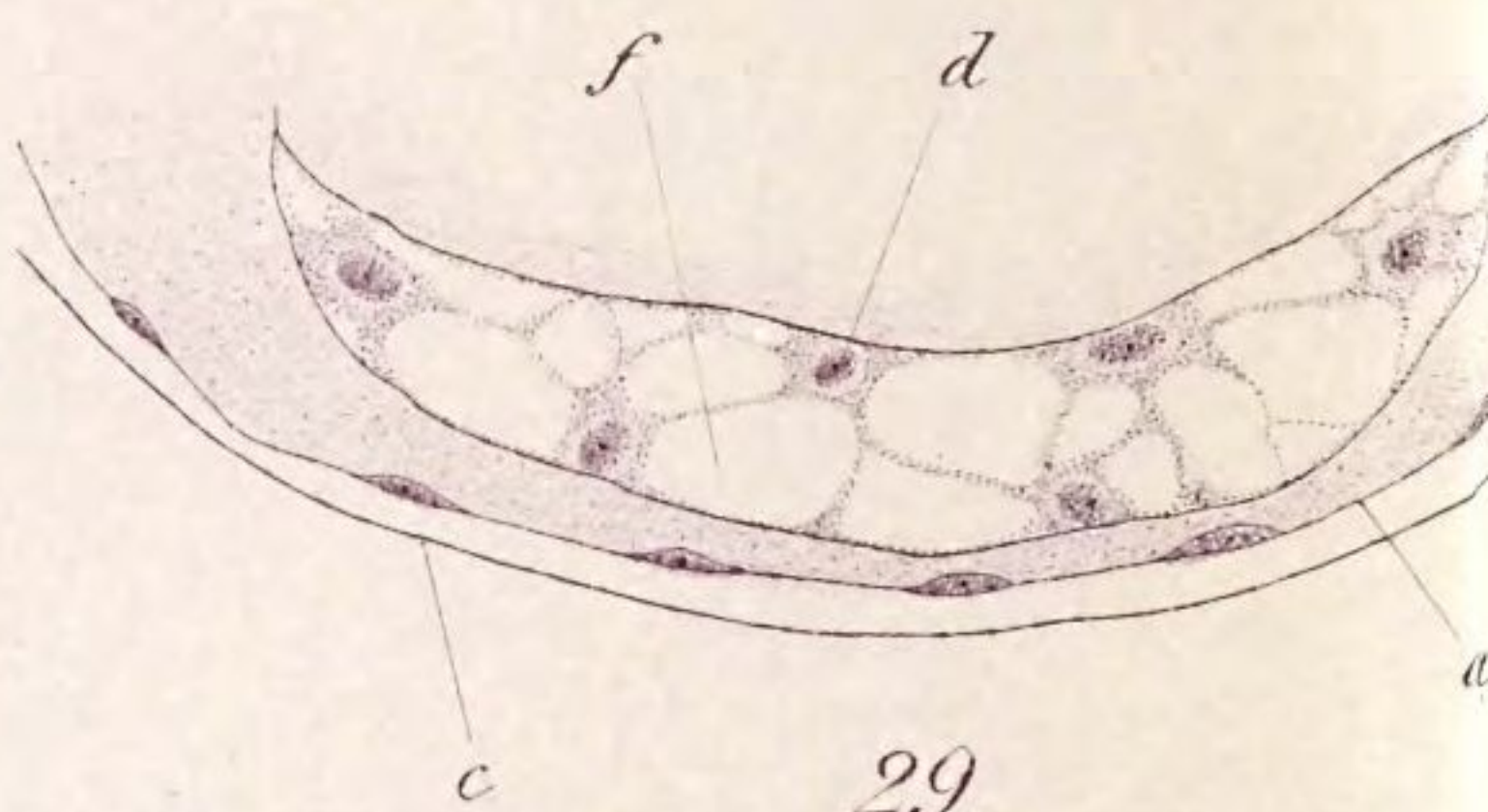
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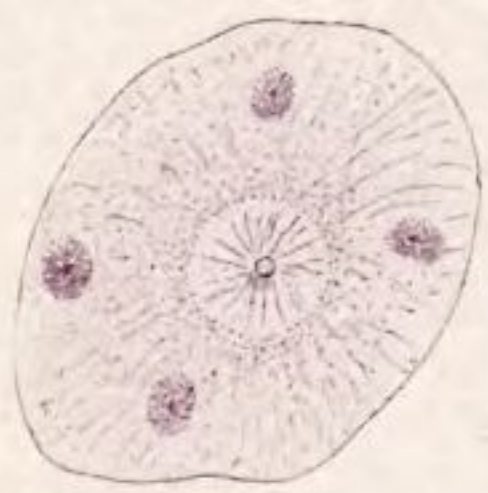
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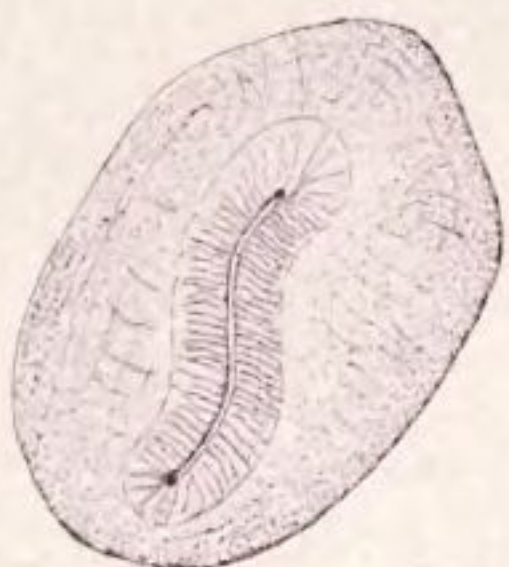
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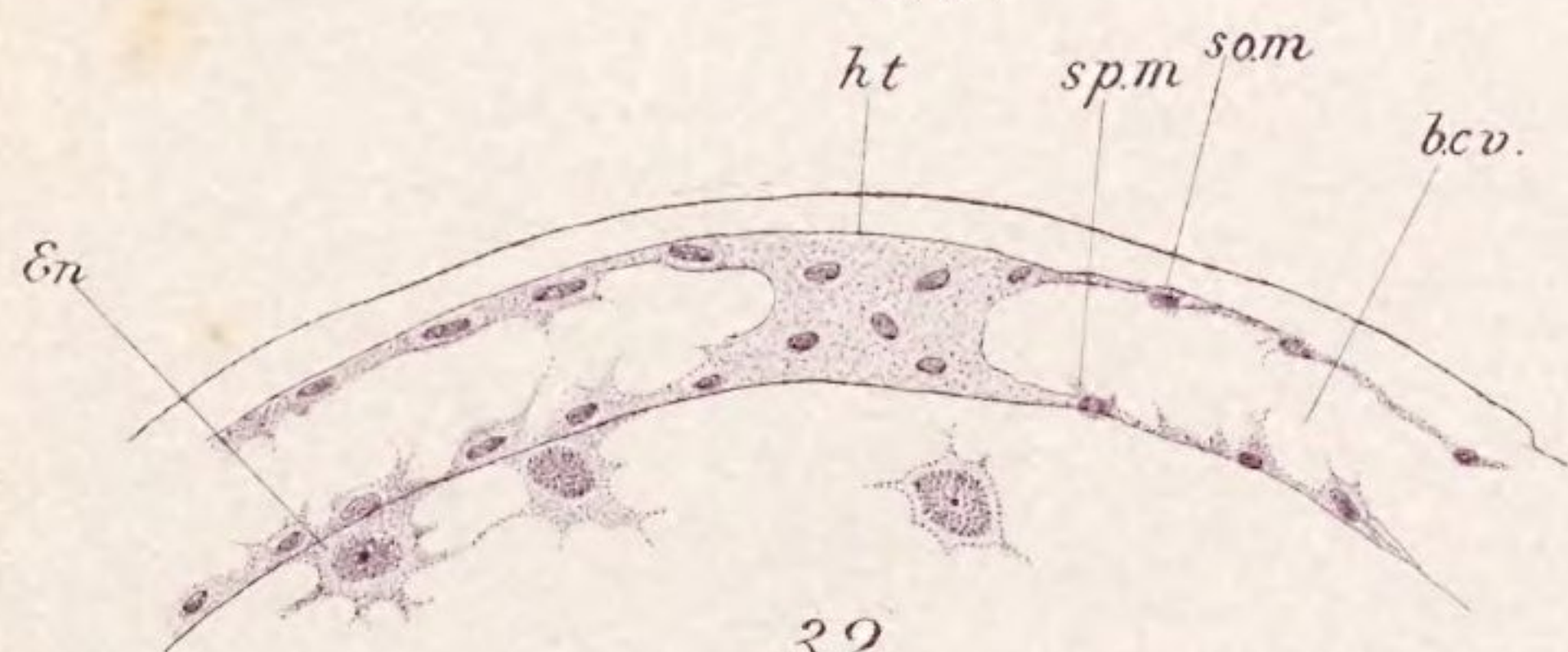
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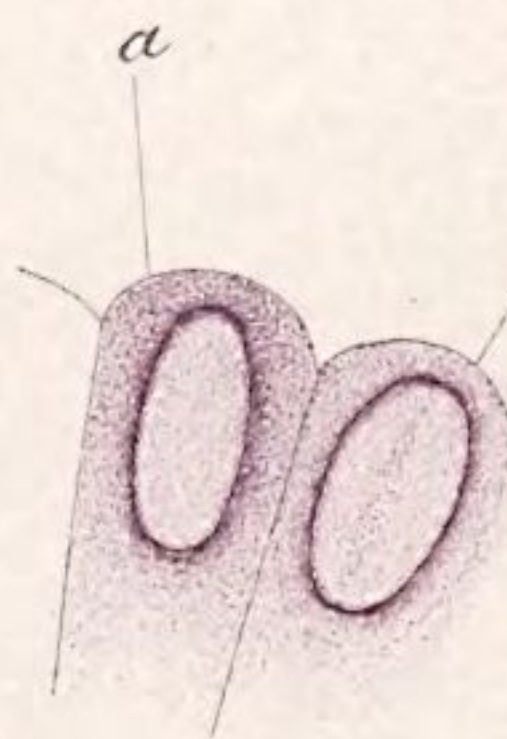
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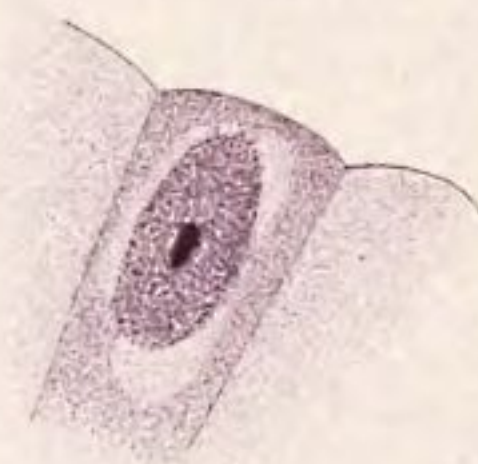
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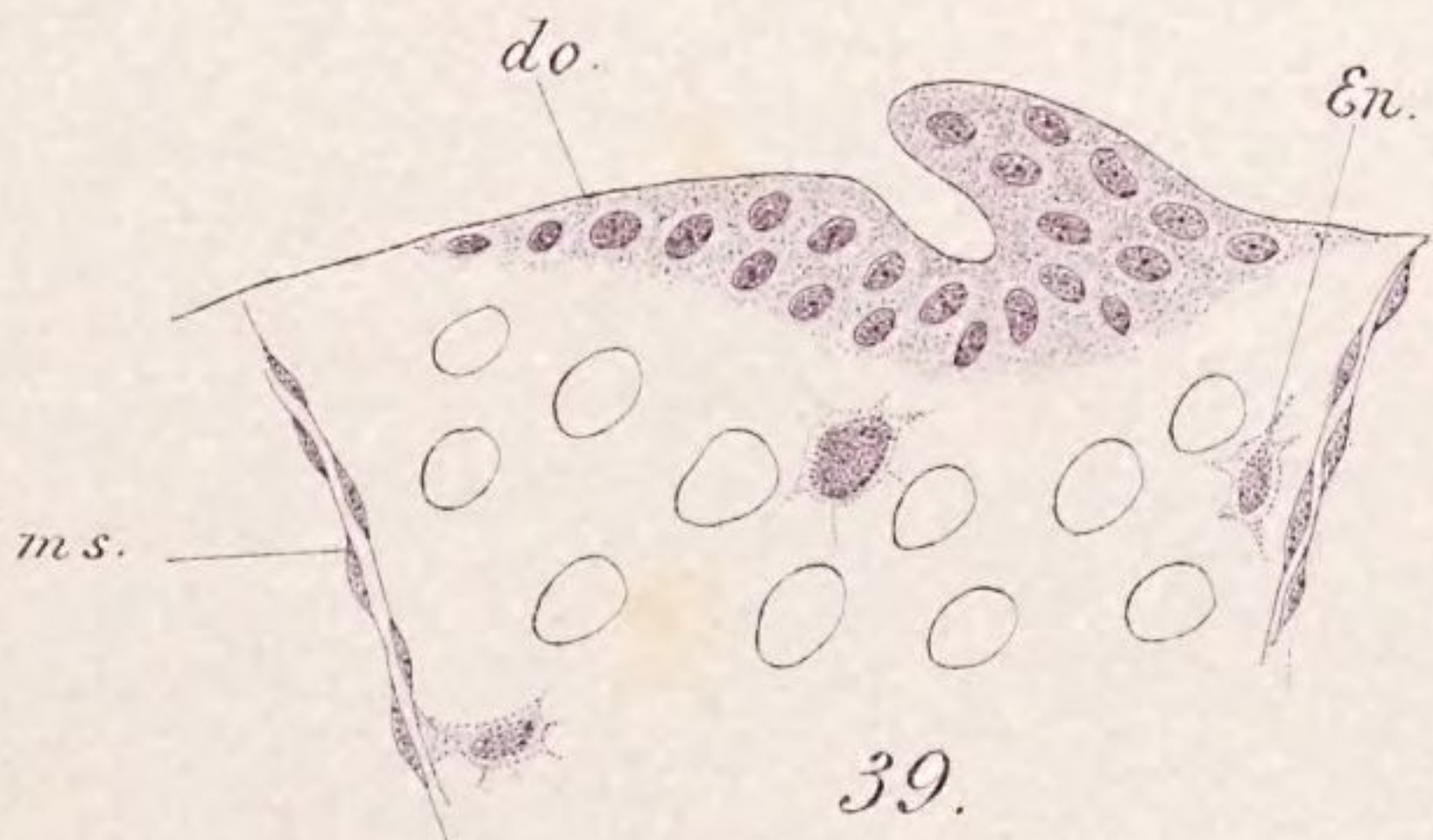
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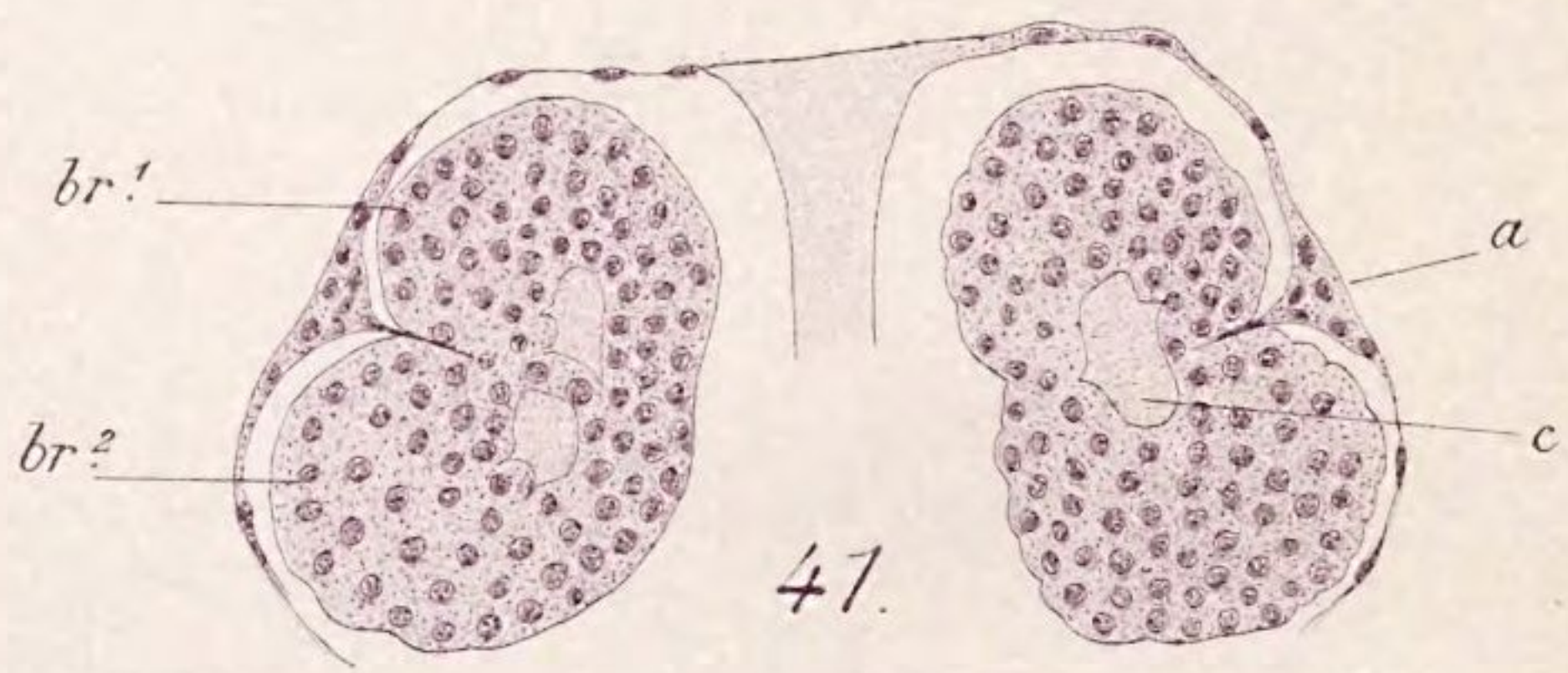
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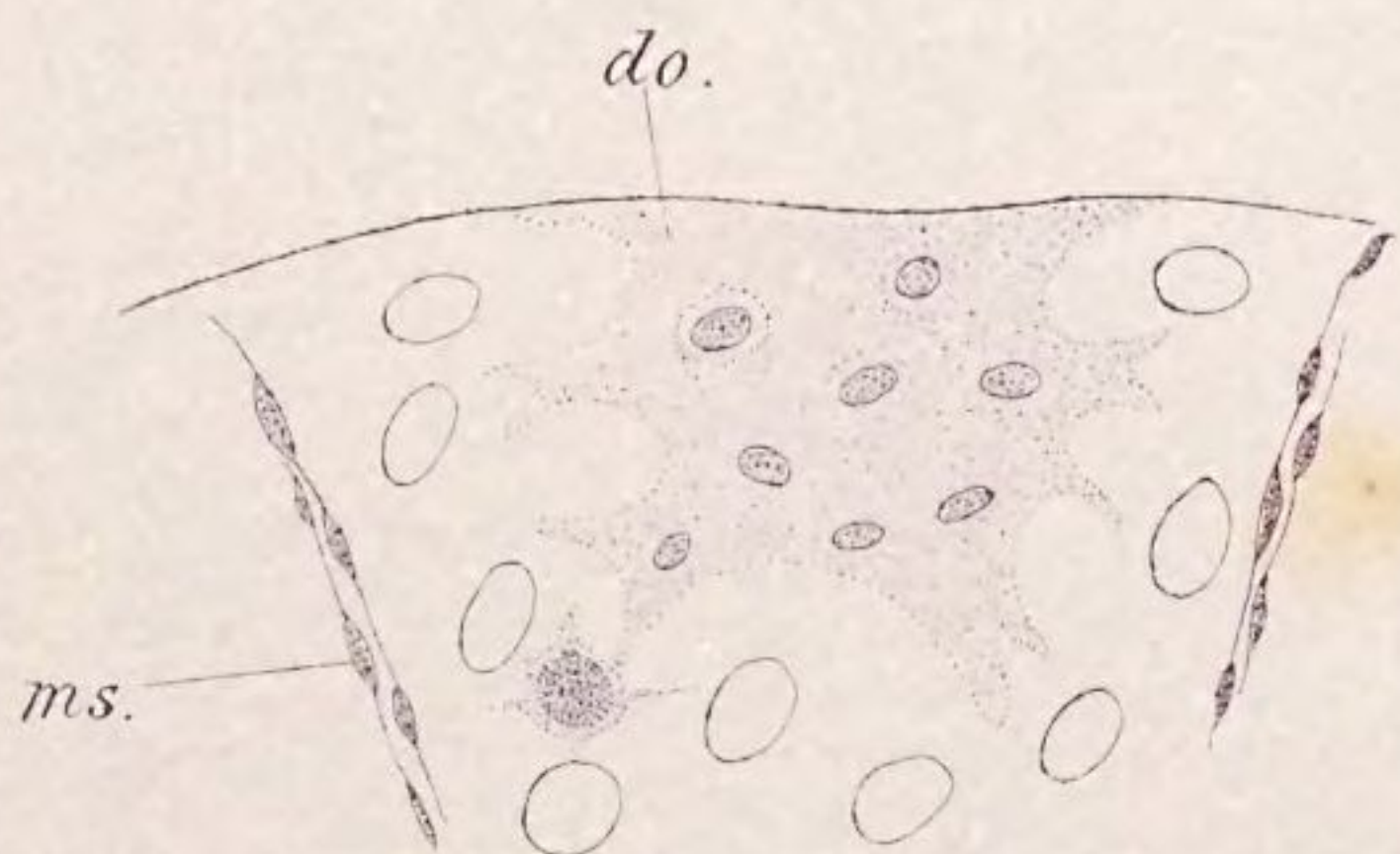
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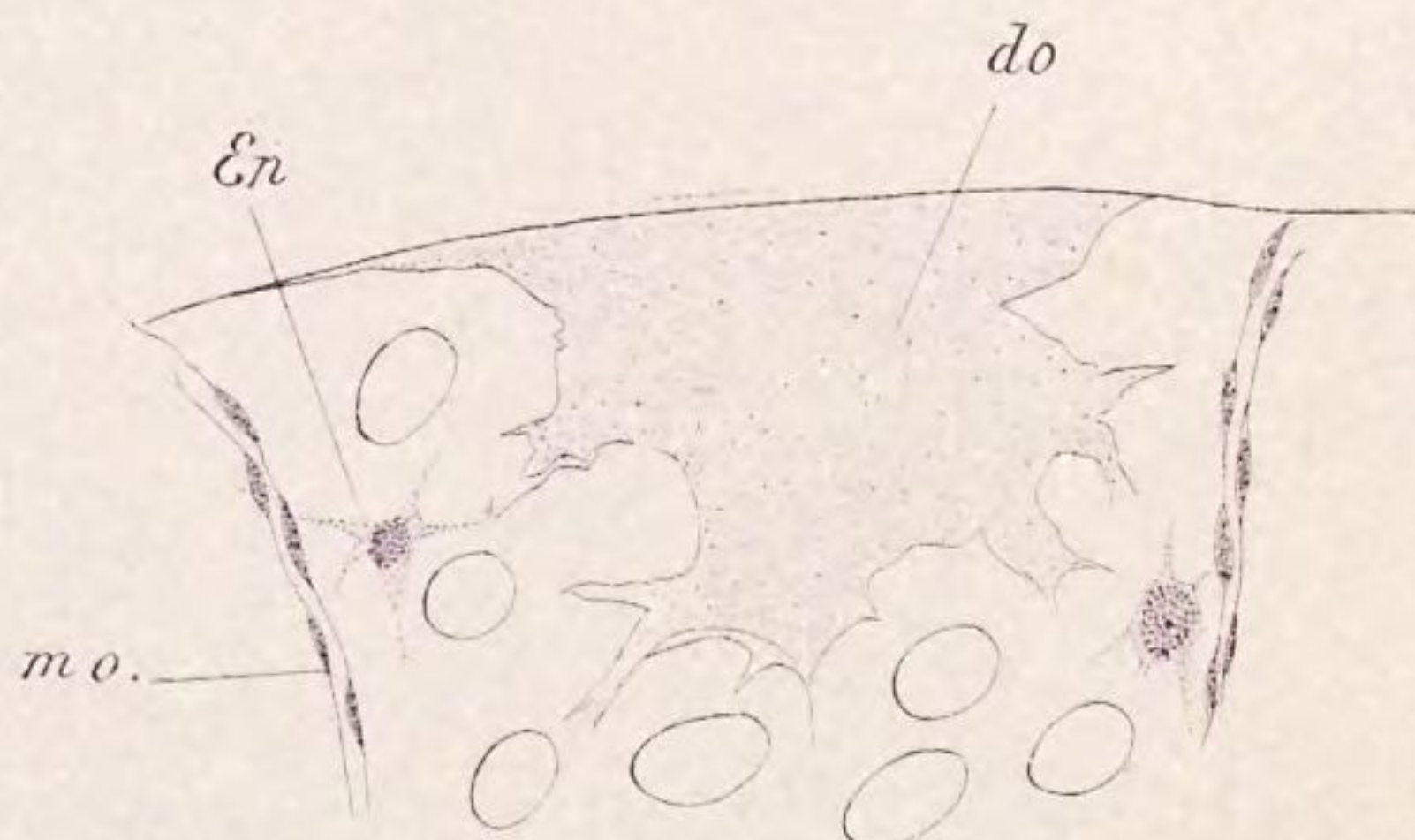
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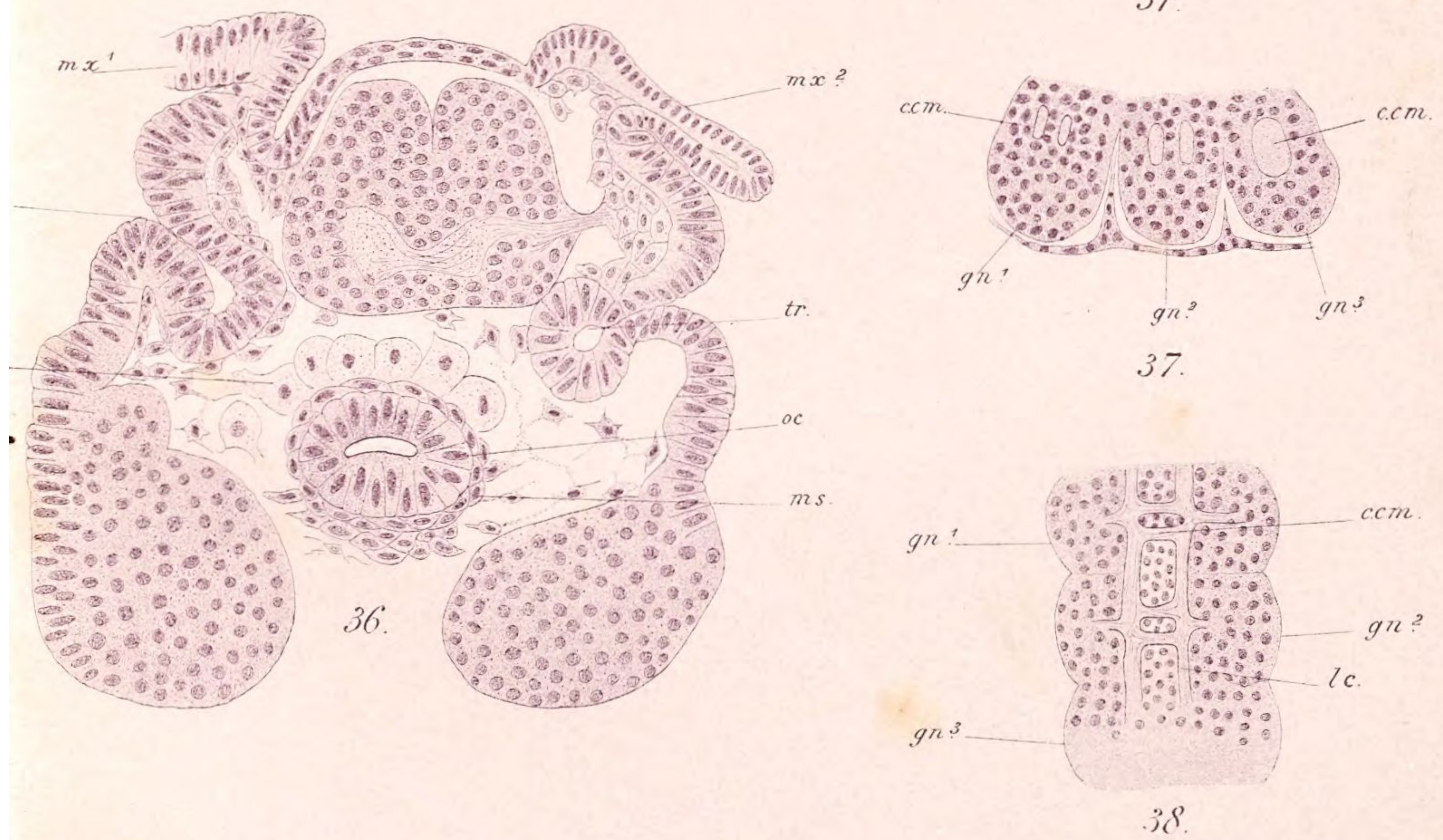
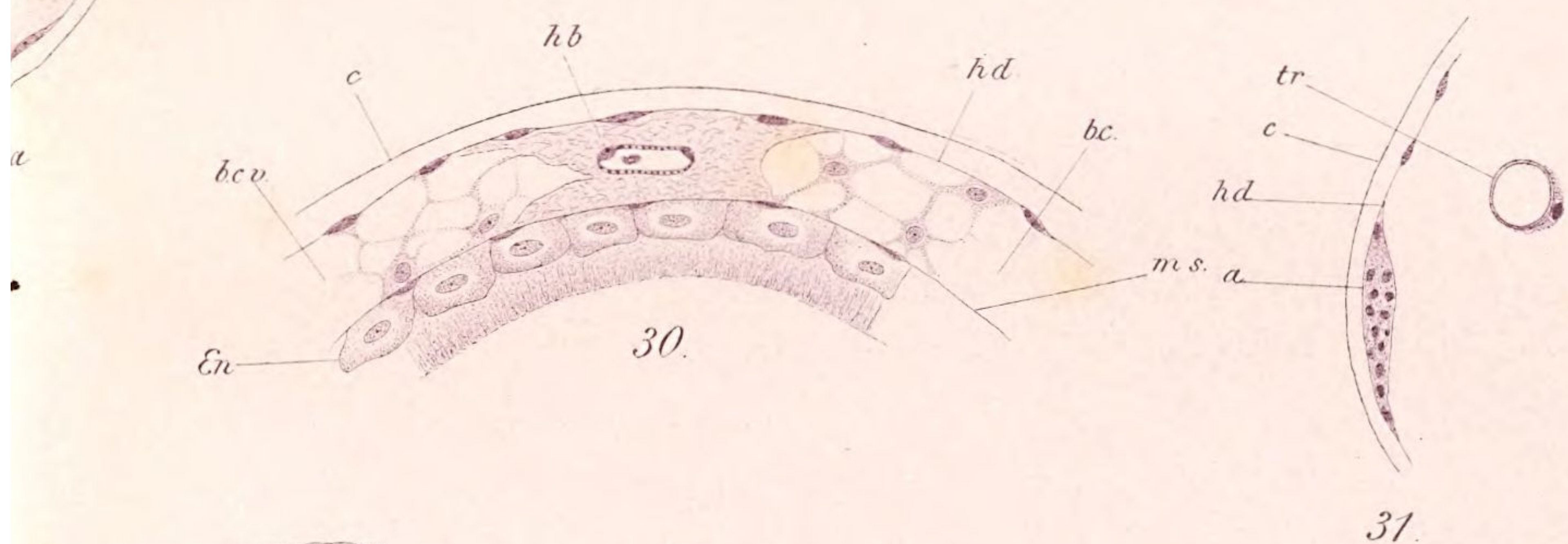
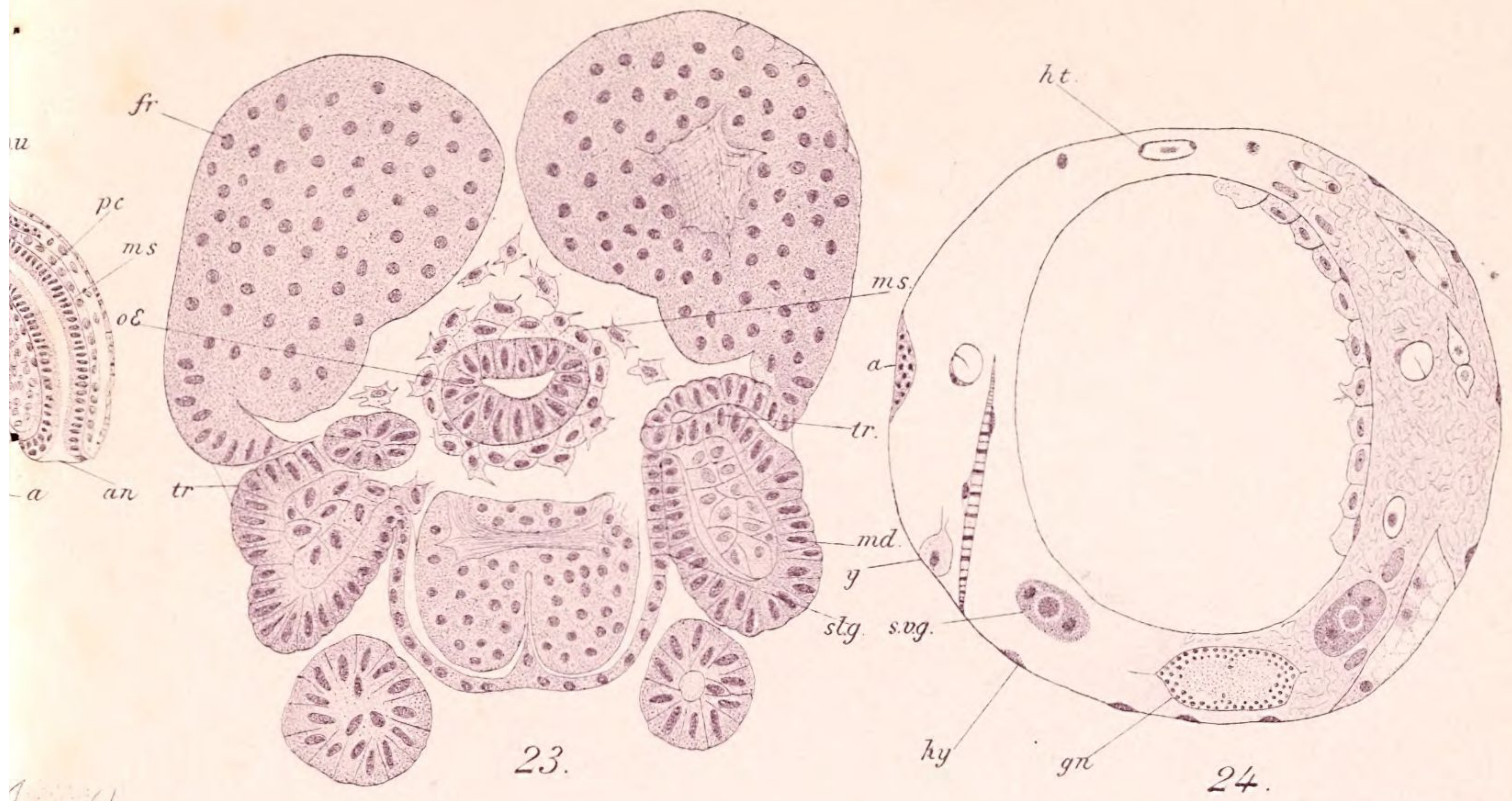
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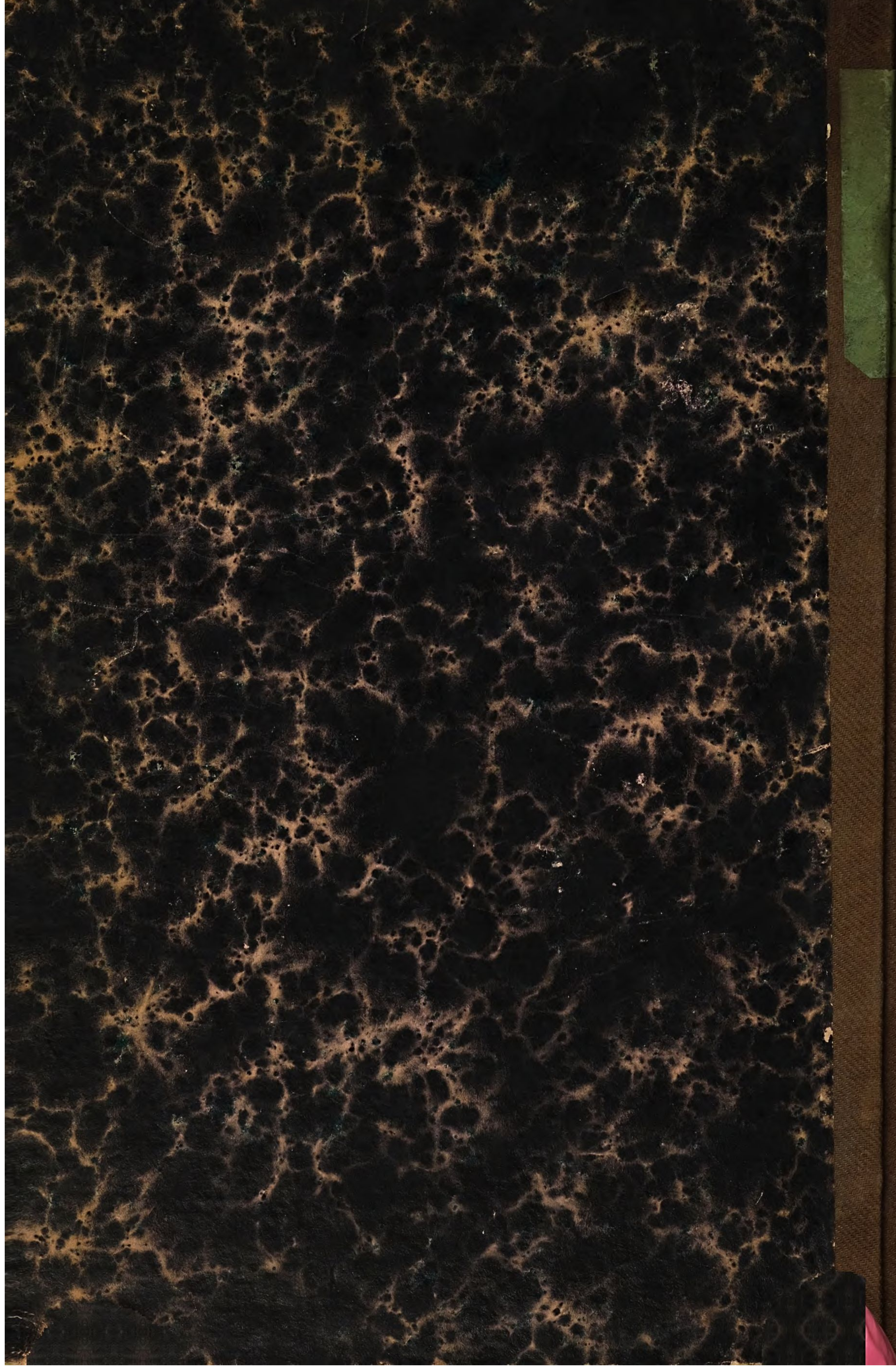


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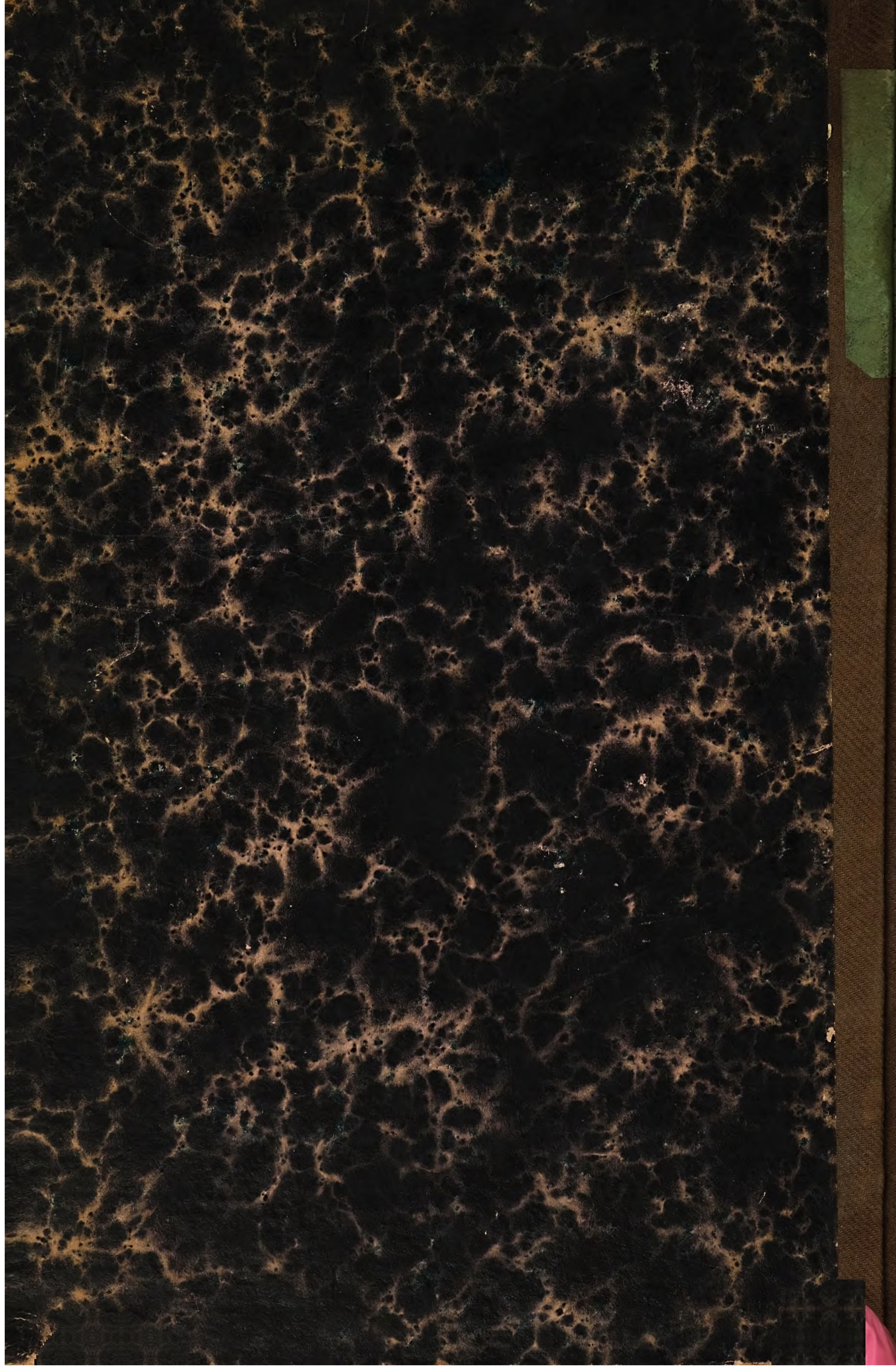


V I T A.

WILLIAM PATTEN was born on the 15th of March, 1861, in Watertown, Massachusetts, United States of America, where he received a common school education. In June, 1879, he passed the entrance examination to the Scientific Department of Harvard University in Cambridge, Massachusetts, where, after four years spent in the study of Modern Languages, Physics, Chemistry, Geology, Palæontology, Physical Geology, Meteorology, Physiology and Morphology of Plants, with special study of Cryptogams, and also of Zoology and Embryology, he passed the final examinations, receiving the degree of "Bachelor of Science." In June, 1883, he was appointed a "Fellow of Harvard University," and was then enabled, in August, 1883, to continue his studies in the "University of Leipzig," under the instruction of Professors Schenk, Zirkel, and Leuckart, receiving special instruction from the latter professor.







Patten, William

The Development of Phryganids with a preliminary note on the development
of Blatta Germanica

London 1884

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